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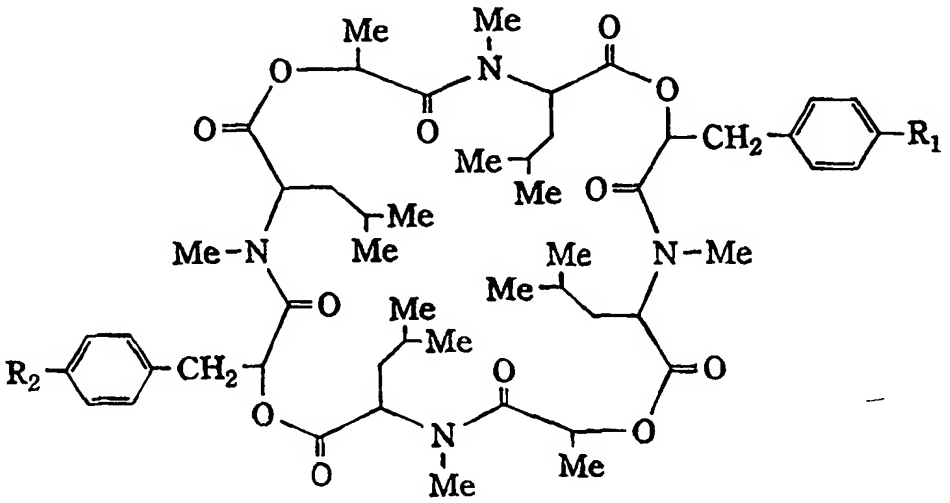

(I)

(57) Abstract

A novel cyclodepsipeptide derivative of formula (I) wherein (i) $R_1$ is hydrogen atom and $R_2$ is a substituted 2–furylmethoxy group, a 3–furylmethoxy group or a 2–thienylmethoxy group, or (ii) both of $R_1$ and $R_2$ are each a 2–furylmethoxy group, a 2–thienylmethoxy group or others, is now provided by chemical syntheses from the fermentatively producible, known PF1022 E or PF1022 H substance. The novel cyclodepsipeptides have excellent and unique anthelmintic activities against parasites of animals and humans.

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# DESCRIPTION

## DERIVATIVES OF CYCLODEPSIPEPTIDE, PF1022 SUBSTANCE

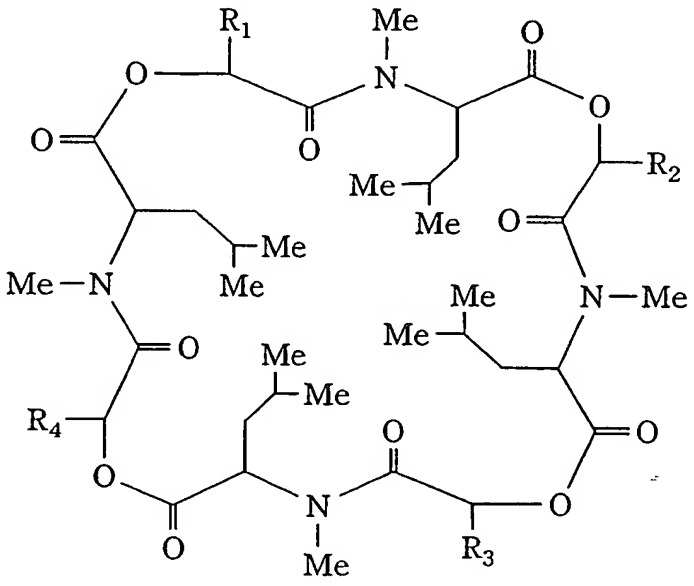
### Technical Field

This invention relates to novel cyclodepsipeptide derivatives having a skeletal structure common to that of PF1022 substance, a known cyclodepsipeptide which is a fermentation product of a microorganism and which has an anthelmintic activity. The novel cyclodepsipeptide derivatives according to this invention have an excellent anthelmintic activity, particularly against organisms of the genus Trichinella. This invention also relates to a vermicidal or anthelmintic composition characterized by comprising the novel cyclodepsipeptide derivative as an active ingredient.

### Background Art

PF1022 substance is a cyclodepsipeptide which was discovered through some investigations of anthelmintically active substances effective against fowl roundworms [see Japanese patent application first publication "Kokai" No. Hei-3-35796, now granted under Japanese patent No. 2608479, US patent No. 5,116,815 of Ohyama et al, European patent application publication No. 0382173 A2, and the "Journal of Antibiotics" 45, page 692 (1992)]. The PF1022 substance is a fermentation product as produced by cultivation of a filamentous fungus, PF1022 strain (deposited under an access number FERM BP-2671 with National Institute of Bioscience

and Human-Technology Agency at Tsukuba-city, Japan in terms of the Budapest Treaty) which belongs to Agonomycetales. The PF1022 strain is now presumed to belong to the genus Xylaria or the genus Rosellinia. The PF1022 substance is one compound embraced by a class of cyclodepsipeptide compounds which are generically represented by the following chemical structural formula (A):



(A)

wherein Me denotes methyl group, and $R_1$, $R_2$, $R_3$ and $R_4$ have such meanings as indicated below.

The cyclodepsipeptide compounds having the general formula (A) above embrace eight compounds which are nominated as PF1022 substance, PF1022 B substance, PF1022 C substance, PF1022 D substance, PF1022 E substance, PF1022 F substance and PF1022 G substance and PF1022 H substance, respectively. The PF1022 substance and PF1022 B to H substances have the under-mentioned meanings for $R_1$ to $R_4$ given in the formula (A), as indicated below:-

PF1022 substance: a compound of the formula (A) where $R_1$ and $R_3$ are each methyl group, and $R_2$ and $R_4$ are each benzyl group

PF1022 B substance: a compound of the formula (A) where $R_1$, $R_2$, $R_3$ and $R_4$ are each benzyl group

PF1022 C substance: a compound of the formula (A) where $R_1$ is methyl group, and $R_2$, $R_3$ and $R_4$ are each benzyl group

PF1022 D substance: a compound of the formula (A) where $R_1$, $R_2$ and $R_3$ are each methyl group, and $R_4$ is benzyl group

PF1022 E substance: a compound of the formula (A) where $R_1$ and $R_3$ are each methyl group, $R_2$ is p-hydroxybenzyl group and $R_4$ is benzyl group

PF1022 F substance: a compound of the formula (A) where $R_1$, $R_2$, $R_3$ and $R_4$ are each methyl group

PF1022 G substance: a compound of the formula (A) where $R_1$, $R_2$ and $R_3$ are each methyl group, and $R_4$ is p-hydroxybenzyl group, and

PF1022 H substance: a compound of the formula (A) where $R_1$ and $R_3$ are each methyl group, and $R_2$ and $R_4$ are each p-hydroxybenzyl group.

The structural formulae of the PF1022, PF1022 B, PF1022 C, PF1022 D, PF1022 E, PF1022 F and PF1022 H substances are given in PCT international publication WO94/19334 (published 1 September 1994) of PCT application No. PCT/JP94/00252 or the corresponding European patent application publication No. 0685419 A1. The PF1022 F and PF1022 H substances were initially nominated as PF1022-002 substance and PF1022-202 substance in the PCT/JP94/00252 application, respectively. The PF1022 G substance is described with its structural formula in the specification of PCT application No. PCT/JP97/02772 (filed 7 August 1997), published under PCT international publication WO98/

The PF1022 substance is the cyclodepsipeptide which is composed of L-N-methylleucine [$(CH_3)_2CHCH_2CH(NHCH_3)COOH$] (abbreviated as H-L-Meleu-OH), D-lactic acid [$CH_3CH(OH)$-COOH] (abbreviated as H-D-Lac-OH) and D-phenyl-lactic acid [$C_6H_5CH_2CH(OH)COOH$] (abbreviated as H-D-PhLac-OH) via ester-bonds and amido-bonds, and which may also be represented by the following formula (A'):

Formula A': Cyclo(L-MeLeu-D-Lac-L-MeLeu-D-PhLac-L-MeLeu-D-Lac-L-MeLeu-D-PhLac).

The cultivation of the filamentous fungus, PF1022 strain results in the production of the PF1022 substance as a main product, and also the production of PF1022 B substance, PF1022 C substance, PF1022 D substance, PF1022 E substance, PF1022 F substance, PF1022 G substance and PF1022 H substance which have the structural formulae as shown above (see also

Japanese patent application first publications "Kokai" Nos. Hei 3-35796, 5-170749 and 6-184126 and Japanese patent application Hei 8-208201), as well as US patent No. 5,116,815, the aforesaid PCT international publication WO/94/19334 and PCT international publication WO98/ of the PCT/JP97/02772 application (filed 7 August 1977).

The PF1022 substance and the PF1022 B to H substances having the anthelmintic activities are distinctly characterized by their specific structure such that all of them have a common cyclodepsipeputide structure as the basic skeleton, that they have, as the side chains of the structure, four N-methyl groups, four isobutyl groups, 0 to 4 methyl groups, 0 to 4 benzyl groups and 0 to 2 p-hydroxybenzyl groups, and also that they contain eight asymmetric carbon atoms in their molecules. Further, it can be presumed that the presence of the 24-membered ring formed by the four ester-bonds and the four amido-bonds as shown in the formula (A) above would play an important role in the expression of their biological activities.

Helmintic infections can cause serious damages to the health of human and animals and also to industries of agriculture and stock breeding. Thus, as an important problem, there exists continuously in the art an outstanding and strong demand to find out and provide such novel and useful substances which possess the anthelmintic activities.

As explained in the above, PF1022 substance was discovered originally as the fermentation product, but some

reports were later issued for methods of preparing the PF1022 substance by chemical syntheses [see Japanese patent application first publication "Kokai" No. 5-320148 and the "Biosci. Biotech. Biochem.", 58, page 1193 (1994)].

It is already known that PF1022 substance and PF1022 B to H substances themselves have very much high anthelmintic activities, but several researcher groups are still working extensive investigations in attempts to produde novel related substance(s) having a higher anthelmintic activity, by utilizing the above known PF1022 related substances as the starting materials.

We, the present inventors, also have continued to make investigations from the initial stage at the time of discovery of the PF1022 substance, in order to produce and find out such novel derivatives which can be derived from PF1022 substance and from PF1022 B to H substances. And we have already succeeded in synthesizing several useful cyclodepsipeptide derivatives (see the PCT international publication WO94/19334; and PCT international publication WO97/11064 (published 27 March 1997) of PCT application No. PCT/JP96/02730 filed 20 September 1996).

Further, the another researcher groups also have disclosed some cyclodepsipeptide derivatives which were produced by total syntheses or other methods(refer to PCT international publication WO93/19053 of PCT/JP93/00286 application, US patent No. 5,514,773, PCT international publication WO95/07272 of PCT/JP94/01446 application, PCT

international publication WO97/11064 of PCT/JP96/02730 application and PCT international publication WO97/20945 of PCT/EP96/05190 application).

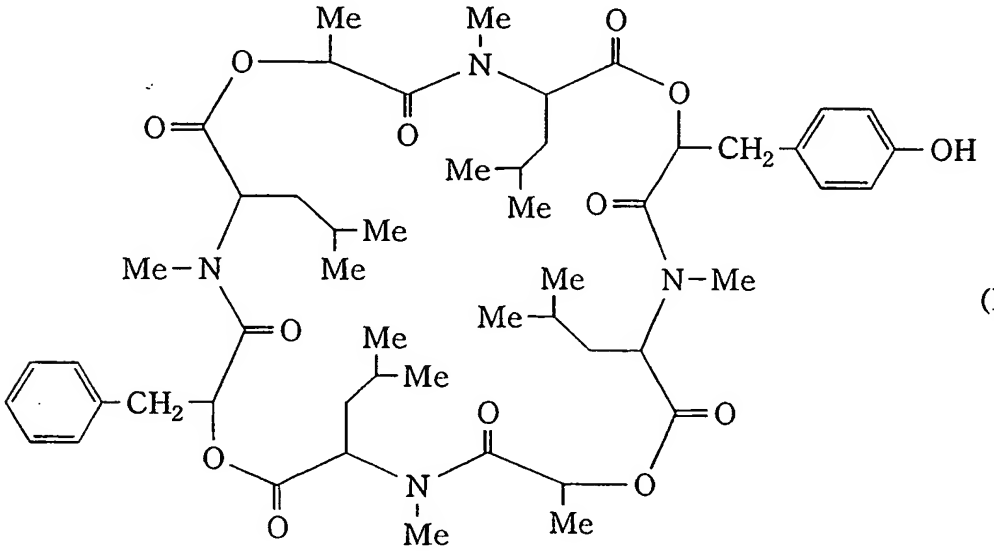
In general, various diseases caused by helminths can bring about serious damages to the health of human and animals and also to industries of agriculture and stock breeding. In account of this situation , it makes continuously an outstanding problem to produce and find out such new substances which are useful as vermicide or anthelmintic having a better anthelmintic activity and which also may easily be produced. With paying attention to the problems as discussed above, we have now made our studies in an attempt to produce and provide novel cyclodepsipeptide derivatives readily, by utilizing the chemical synthetic processes with starting from the fermention product(s), and also we have made our investigations in an attempt to find out some characteristic anthelmintic activities in said novel cyclodepsipeptide derivatives as produced.

As described above, the present inventions have carried out investigations for the purposes of producing such novel derivatives of PF1022 substance and PF1022 B to H substances by means of chemical syntheses with starting from the PF1022 substance, and PF1022 B to H substances so that there can be produced such novel cyclodepsipeptide derivatives which would exhibit higher anthelmintic activities than those of the known PF1022 and PF1022 B to H substances. As a result of our investigations, the present

inventors have now succeeded in sythesizing and providing novel cyclodepsipeptides, namely such novel PF1022 derivatives which have superior anthelmintic activities to those of the known PF1022 related compounds and can have particularly high anthelmintic activities against helminths of the genus Trichinella.

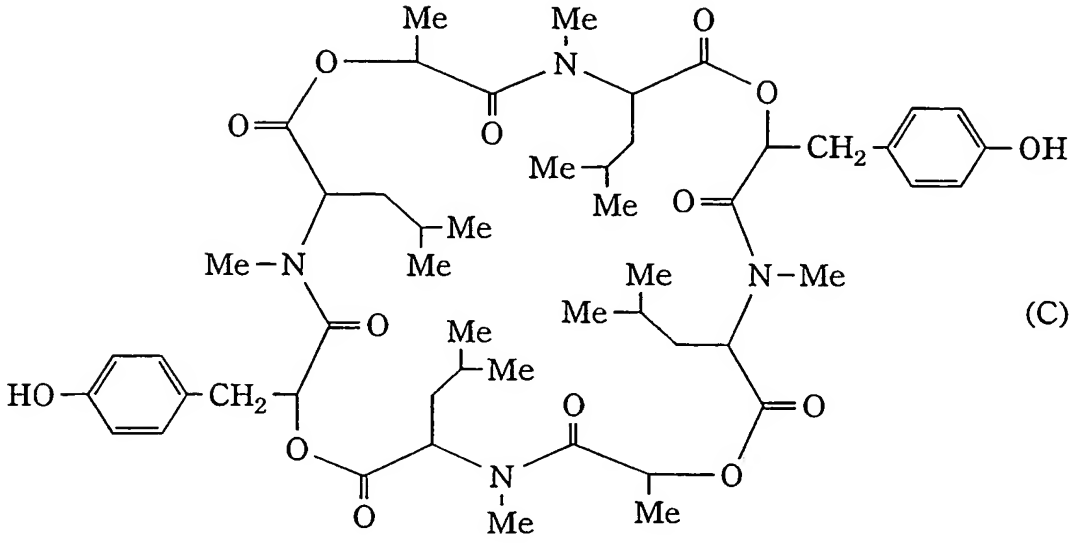
Thus, the present inventors have now proceeded our investigations with taking notice of the D-phenyllactic acid moiety which is one of the components of forming the PF1022 substances. More particularly, the inventors now have produced novel cyclodepsipeptides, namely novel PF1022 derivatives having strong anthelmintic activity specially against organisms of the genus Trichinella, by means of chemical synthetic processes which comprise using as the starting compound PF1022 E substance or PF1022 H substance.

In details, the PF1022 E substance is represented by the structural formula (B):



(B)

the PF1022 H substance is represented by the structural formula (C)



(C)

Disclosure of the Invention

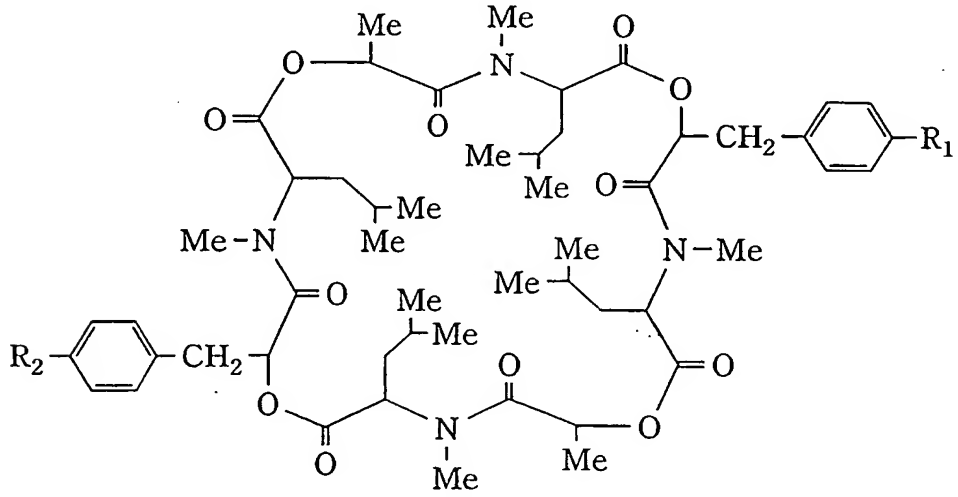
An object of this invention is to provide novel cyclodepsipeptide derivatives of PF1022 substance. Another object of this invention is to provide an anthelmintic composition comprising the novel PF1022 derivative as an active ingredient.

The PF1022 E substance and PF1022 H substance, which are used as the starting materials for sythesizing said novel cyclodepsipeptide derivatives of PF1022 substance, are the by-products obtained concurrently in the fermentative preparation of PF1022 substance. The PF1022E substance, and PF1022 H substance by themselves have anthelmintic activity against animal parasites and also are much more interesting in their chemical structures in comparison with PF1022 substance. Thus, PF1022 E substance has a hydroxyl group at the para-position of one benzene ring of the two benzene rings

present in the molecule, and PF1022 H substance has hydroxyl groups at both the para-position of the said two benzene rings. We have now taken notice of the fact that the presence of such functional hydroxyl group(s) would further increase the possibility of chemically modifying these substances. Accordingly, we have carried out chemical syntheses of the novel cyclodepsipeptide derivatives of PF1022 substance by applying chemical conversions to said functional groups of the PF1022 E or H substance.

The present inventors have now thus succeeded in producing and providing a series of new cyclodepsipeptide derivatives which may be represented collectively by the general formula (I) shown below.

According to a first aspect of this invention, therefore, there is provided, as new compounds, a cyclodepsipeptide represented by the following general formula (I)



(I)

wherein Me denotes methyl group, and (i) $R_1$ is a hydrogen atom and $R_2$ is a 2-furylmethoxy group having one, two or three

substituents on the furan ring, a 3-furylmethoxy group optionally having one, two or three substituents on the furan ring, a 2-thienylmethoxy group optionally having one, two or three substituents on the thiophene ring or a 3-thienylmethoxy group optionally having one, two or three substituents on the thiophene ring, or alternatively (ii) both of $R_1$ and $R_2$ are identical to each other and are each a 2-furylmethoxy group optionally having one, two or three substituents on the furan ring, a 3-furylmethoxy group optionally having one, two or three substituents on the furan ring, a 2-thienylmethoxy group optionally having one, two or three substituents on the thiophene ring or a 3-thienylmethoxy group optionally having one, two or three substituents on the thiophene ring, or a non-toxic salt of said cyclodepsipeptide of the formula (I).

When the novel cyclodepsipeptide of the general formula (I) according to this invention is basic in its nature owing to the imino groups and/or amino groups contained therein, said cyclodepsipeptide can form a salt with an acid. Therefore, the non-toxic salt of the cyclodepsipeptide of the formula (I) includes a pharmaceutically acceptable acid addition salt with a pharmaceutically acceptable inorganic acid such as hydrochloric acid, sulfuric acid and phosphoric acid, or a pharmaceutically acceptable organic acid such as acetic acid, citric acid, lactic acid, methanesulfonic acid and the like.

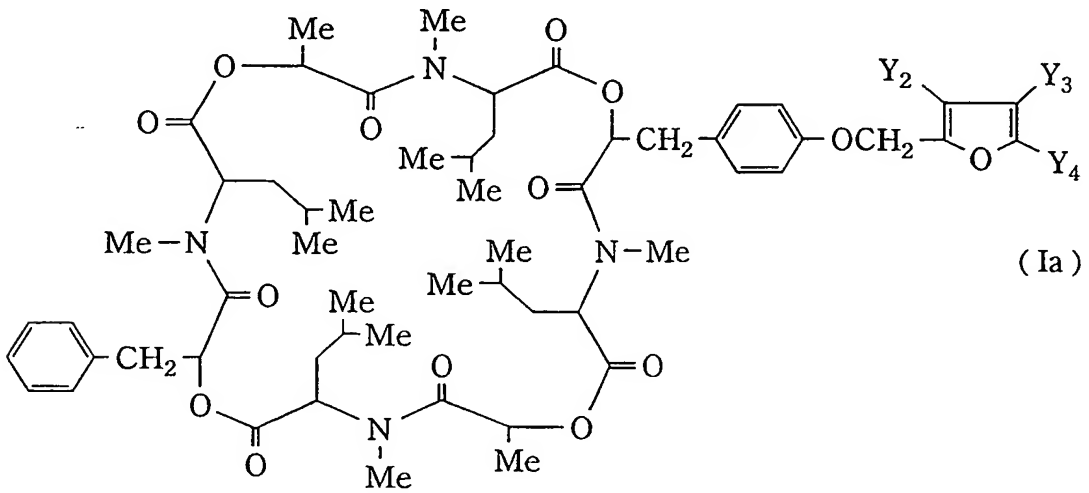
In the cyclodepsipeptide of the formula (I) according

to this invention, the one, two or three substituents which may be born on the furan ring or thiophene ring of the cyclodepsipeptide may each independently be an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$) alkyl group substituted by an N-mono-($C_1$-$C_6$)alkylamino group, an N,N-di-($C_1$-$C_6$) alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$)alkoxycarbonyl group.

Best Embodiments for working the Invention

The cyclodepsipeptide of the general formula (I) according to the first aspect of this invention includes four classes of the cyclodespsipeptides which are respectively represented by the following formulae (Ia), (Ib), (Ic) and (Id) and which are as follows:-

(1) A cyclodepsipeptide represented by the following general formula (Ia)

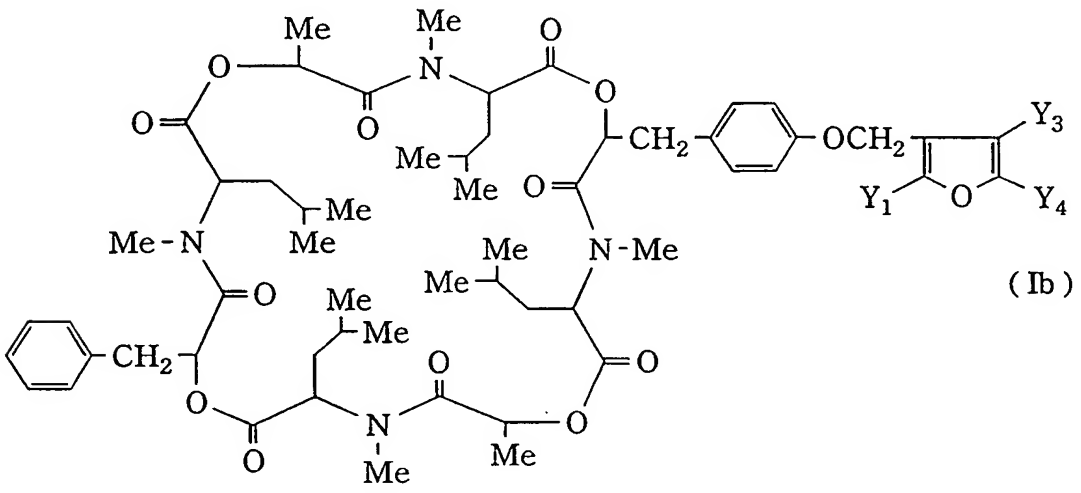


(Ia)

wherein Me is methyl group, and $Y_2$, $Y_3$ and $Y_4$ are each independently a substituent chosen from an unsubstituted

linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$) alkyl group substituted by an N-mono-($C_1$-$C_6$) alkylamino group, an N,N-di-($C_1$-$C_6$) alkylamino group, a ($C_1$-$C_6$) alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$) alkoxycarbonyl group.

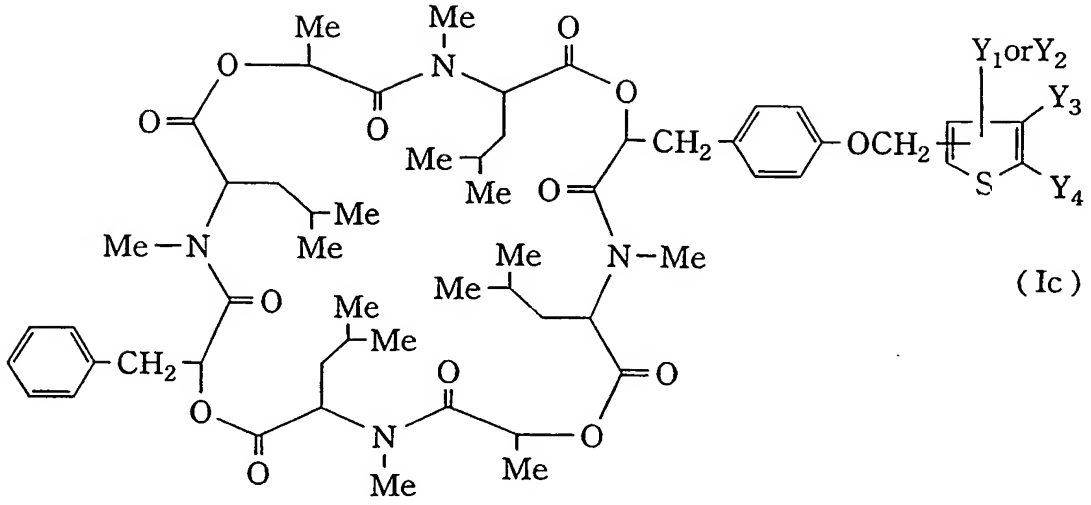
(2) A cyclodepsipeptide represented by the following general formula (Ib)



(Ib)

wherein Me is methyl group, and $Y_1$, $Y_3$ and $Y_4$ are each independently a hydrogen atom or a substituent chosen from an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$) alkyl group substituted by an N-mono-($C_1$-$C_6$) alkylamino group, an N,N-di-($C_1$-$C_6$) alkylamino group, a ($C_1$-$C_6$) alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$) alkoxycarbonyl group.

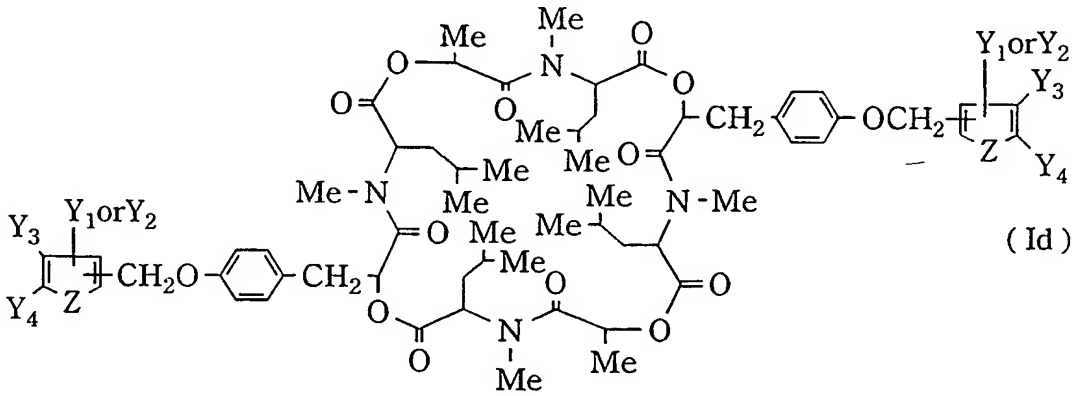
(3) A cyclodepsipeptide represented by the following general formula (Ic)



(Ic)

wherein Me is methyl group, and the existing $Y_1$ or $Y_2$, $Y_3$ and $Y_4$ are each independently a hydrogen atom or a substituent chosen from an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$)alkyl group substituted by an N-mono-($C_1$-$C_6$)alkylamino group, an N,N-di-($C_1$-$C_6$)alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$) alkoxycarbonyl group.

(4) A cyclodepsipeptide represented by the following general formula (Id)



(Id)

wherein Me is methyl group, Z is an oxygen atom or a sulfur atom, and the existing $Y_1$ or $Y_2$, $Y_3$ and $Y_4$ are each independently a hydrogen atom or a substituent chosen from an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$)alkyl group substituted by an N-mono-($C_1$-$C_6$)alkylamino group, an N,N-di-($C_1$-$C_6$) alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$)alkoxycarbonyl group.

Particular examples of the novel cyclodepsipeptide of the general formula (I) as the derivatives of PF1022 substance provided according to this invention are the following compounds:-

1. Cyclo[MeLeu-Lac-MeLeu-(2-furylmethoxy)PhLac]$_2$ (Compound Code No.: PF1022-888 substance)

2. Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(3-furylmethoxy)PhLac] (Compound Code No.: PF1022-356 substance)

3. Cyclo[MeLeu-Lac-MeLeu-(3-furylmethoxy)PhLac]$_2$ (Compound Code No.: PF1022-357 substance)

4. Cyclo[MeLeu-Lac-MeLeu-PhLac MeLeu-Lac-MeLeu-(3-thienylmethoxy)PhLac] (Compound Code No.: PF1022-352 substance)

5. Cyclo[MeLeu-Lac-MeLeu-(2-thienylmethoxy)PhLac]$_2$ (Compound Code No.: PF1022-353 substance)

6. Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(5-ethoxycarbonyl-2-furylmethoxy)PhLac] (Compound Code No.: PF1022-358 substance)

7. cyclo[MeLeu-Lac-MeLeu-(5-ethoxycarbonyl-2-furylmethoxy)PhLac]$_2$ (Compound Code No.: PF1022-359 substance)

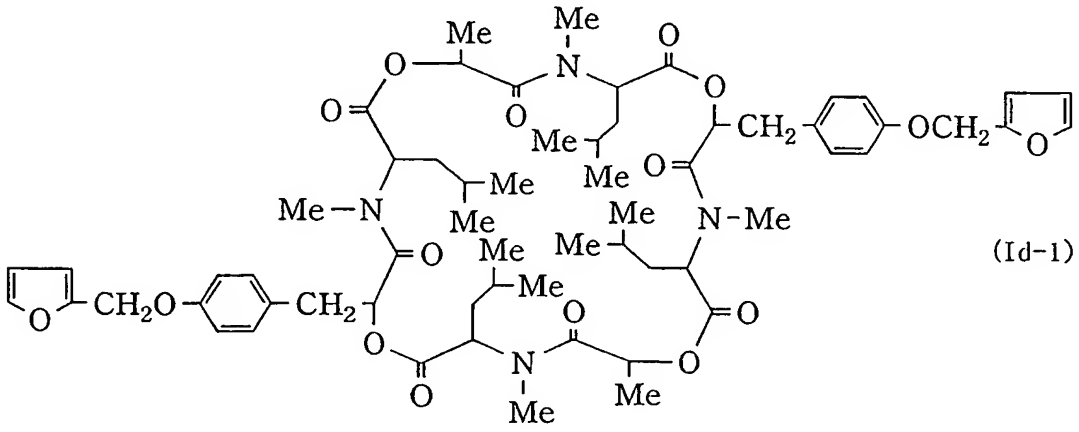
8. Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(5-methyl-2-furylmethoxy)PhLac] (Compound Code No.: PF1022-360 substance)

9. Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(5-ethyl-2-furylmethoxy)PhLac] (Compound Code No.: PF1022-362 substance)

10. Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(4,5-dimethyl-2-furylmethoxy)PhLac] (Compound Code No.: PF1022-364 substance)

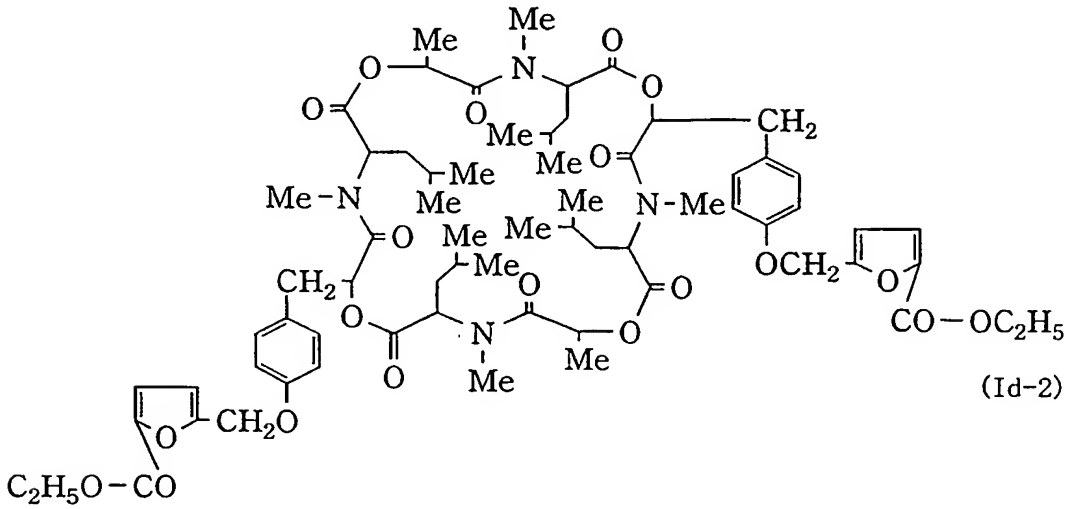
11. Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(5-(N,N-dimethylaminomethyl)-2-furylmethoxy)PhLac] (compound Code No.: PF1022-366 substance).

Among the particular substances referred to in the above, Cyclo[MeLeu-Lac-MeLeu-(2-furylmethoxy)PhLac]$_2$ (Compound Code Number: PF1022-888 substance) is particularly preferred. This PF1022-888 substance is a cyclodepsipeptide represented by the following formula (Id-1)



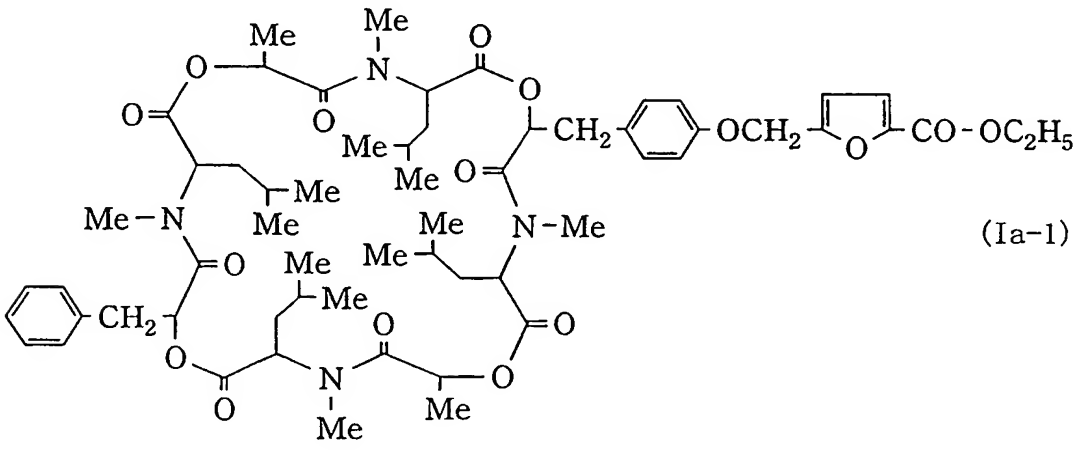
(Id-1)

Further, preferred are the PF1022-359 substance which is a cyclodepsipeptide represented by the following formula (Id-2)



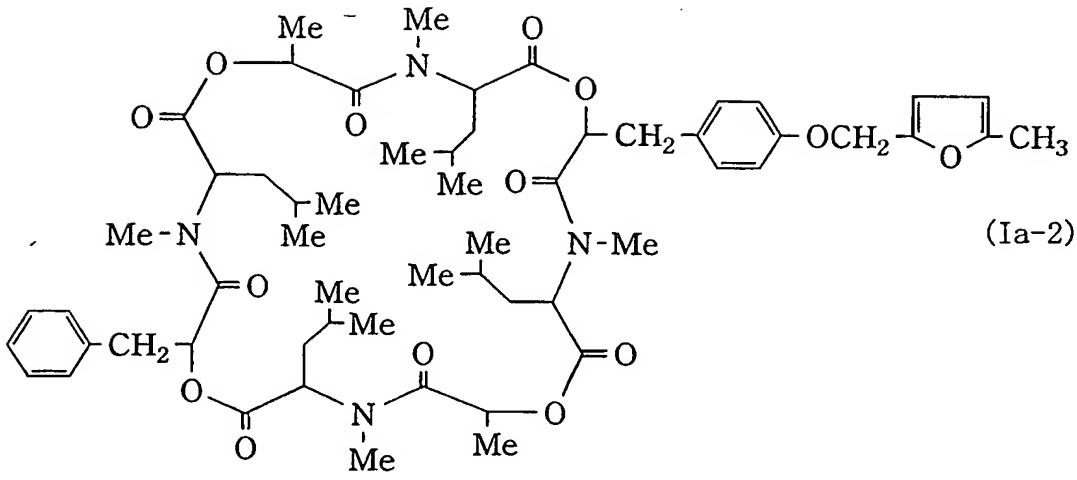
(Id-2)

as well as the PF1022-358 substance which is a cyclodepsipeptide represented by the following formula (Ia-1)



(Ia-1)

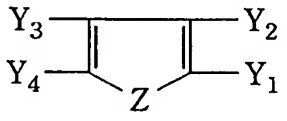
and the PF1022-360 substance which is a cyclodepsipeptide represented by the following formula (Ia-2)



(Ia-2)

Next, methods for preparation of the cyclodepsipeptide derivatives of the general formula (I) according to this invention are described.

In general, the cyclodepsipeptide derivatives of the general formula (I) according to this invention may be synthesized by such a method which comprises reacting the PF1022 E substance of the formula (B) or the PF1022 H substance of the formula (C) shown hereinbefore with a furan compound or a thiophene compound represented by the following general formula (II)



(II)

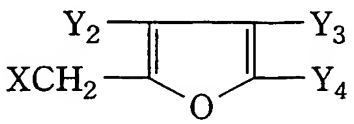
wherein Z is an oxygen atom or a sulfur atom, and one of $Y_1$, $Y_2$, $Y_3$ and $Y_4$ is a halogenomethyl group, particularly chloromethyl, bromomethyl or iodomethyl group, or alternatively a hydroxymethyl group, and one, two or three of the remainder of $Y_1$, $Y_2$, $Y_3$ and $Y_4$ other than the halogenomethyl group or the hydroxymethyl group is or are

a hydrogen atom or such substituent(s) which can bond to the furan ring or the thiophene ring of the compound of the general formula (II) but are not limited to a special one, and which may be identical to each other or may be different from each other.

The substituent(s), which may exist as one, two or three substituents for $Y_1$, $Y_2$, $Y_3$ and $Y_4$ on the furan ring or thiophene ring of the furan compound or thiophene compound of the general formula (II), may each independently be a linear or branched lower ($C_1$-$C_6$)alkyl group, unsubstituted or substituted by an N-mono-($C_1$-$C_6$)alkylamino group, an N,N-di-($C_1$-$C_6$)alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or be a linear or branched lower ($C_1$-$C_6$) alkoxycarbonyl group.

The furan compound or thiophene compound of the formula (II) useful for the synthetic production of the new cyclodepsipeptide of the formula (I) may approximately be classified into and embraces the following three types of the compounds of the formulae (IIa), (IIb) and (IIc) shown below.

(i) A 2-halomethylfuran compound or 2-hydroxymethylfuran compound of the formula (IIa)

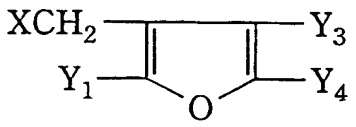



(IIa)

wherein X is a chlorine, bromine or iodine atom or X is a hydroxyl group, and $Y_2$, $Y_3$ and $Y_4$ are each independently a

hydrogen atom or such a substituent as indicated in the above, provided that at least one of $Y_2$, $Y_3$ and $Y_4$ is not the hydrogen atom.

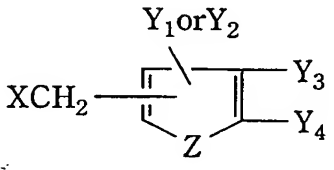
(ii) A 3-halomethylfuran compound or 3-hyroxymethylfuran compound of the formula (IIb)



(IIb)

wherein X is a chlorine, bromine or iodine atom or a hydroxyl group as defined in the above, and $Y_1$, $Y_3$ and $Y_4$ are each independently a hydrogen atom or a such a substituent as indicated in the above.

(iii) A 2- or 3-halomethylfuran compound or 2- or 3-halomethylthiophene compound or 2- or 3-hydroxymethylfuran compound or 2- or 3-halomethylthiophene compound of the formula (IIc)



(IIc)

wherein X is as defined in the above, Z is an oxygen atom or a sulfur atom, and such one of $Y_1$ and $Y_2$ which is neither the halomethyl group nor the hydroxymethyl group, as well as $Y_3$ and $Y_4$ are each independently a hydrogen atom or such a substituent as indicated in the above.

When the starting material, PF1022 E substance of the formula (B) or the starting material, PF1022 H substance of

the formula (C) is reacted with the furan or thiophene compound of the formula (II) which contains the halomethyl group as $Y_1$ or $Y_2$, more particularly the furan compound or thiophene compound of the formula (IIa), (IIb) or (IIc) where the group $-CH_2X$ is the halomethyl group, particularly chloromethyl, bromomethyl or iodomethyl group, the starting substance of the formula (B) or (C) may be reacted with the reactant furan compound or thiophene compound of the formula (II), more particularly the compound of the formula (IIa), (IIb) or (IIc) having the halomethyl group for $-CH_2X$, in an inert organic solvent in the presence of a base which can serve as a hydrogen halide scanvenger.

When the furan compound or thiophene compound of the formula (II), particularly the compound of the formula (IIa), (IIb) or (IIc), which is containing chloromethyl group or bromomethyl group for $-CH_2X$, is used as a reactant to be reacted with PF1022 E or H substance, the condensation reaction as intended can often be made to proceed more smoothly by adding a metal iodide or tetraalkylammonium iodide to the reaction mixture. As the inert organic solvent for the reaction, there may be mentioned ethers such as ethyl ether, isopropyl ether, tetrahydrofuran (THF), 1,4-dioxane, etc.; ketones such as acetone,2-butanone etc.; a halogenated hydrocarbon solvent such as dichloromethane, chloroform, etc.; N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), etc. They are used singly or in combination. As the above-mentioned base, there may be used an inorganic base such as

potassium t-butoxide, sodium hydride, potassium carbonate, sodium carbonate, cesium carbonate, etc.; and an organic base such as triethylamine, 1,8-diaza-bicyclo [5.4.0]-7-undecene, etc. Appropriate reaction temperature may suitably vary with the nature of the furan or thiophene compound of formula(II), the solvent and base as used. A reaction temperature in a range of room temperature to 90°C is preferred to give good results in most cases.

When the furan compound or thiophene compound of the formula (II), particularly the compound of the formula (IIa), (IIb) or (IIc), which is containing the hydroxymethyl group for $-CH_2X$, is used as a reactant to be reacted with PF1022 E or H substance, the condensation reaction as intended can be conducted by so-called "Mitsunobu" reaction to afford the target condensation product in a facile manner. Illustrativekly, the condensation reaction between PF1022 E or H substance and the furan compound or thiophene compound containing the hydroxymethyl group for $-CH_2X$ may be carried out an inert organic solvent with using as a condensation reagent a diazocarboxylic acid derivative such as diethyl azodicarboxylate, azodicarbonyl dipiperidine, etc.; or a trivalent phosphorus compound such as triphenylphosphine, tributylphosphine, etc. As the organic solvent, THF and the like may be used.

Thus, in detail, the cyclodepsipeptide of the formula (Ia) according to the first aspect of this invention can be produced by a process comprising reacting 1 molar proportion

of PF1022 E substance with 1 molar proportion or a slightly excessive proportion of the 2-halomethylfuran compound or 2-hydroxymethylfuran compound of the above formula (IIa) in the way as described above.

The cyclodepsipeptide of the formula (Ib) can be produced by a process comprising 1 molar proportion of PF1022 E substance with 1 molar proportion or a slightly excessive proportion of the 3-halomethylfuran compound or 3-hydroxymethylfuran compound of the above formula (IIb) in the way as described above.

Further, the cyclodepsipeptide of the formula (Ic) can be produced by a process comprising reacting 1 molar proportion of PF1022 E substance with 1 molar proportion or a slightly excessive proportion of the 2- or 3-halomethylthiophene compound or 2- or 3-hydroxymethylthiophene compound according to the above formula (IIc) in the way as described above. The cyclodepsipeptide of the formula (Id) according to this invention can also be produced by a process comprising reacting 1 molar proportion of PF1022 H substance with 2 molar proportions or slightly excessive proportions of the 2- or 3-halomethylfuran compound or 2- or 3-halomethylthiophene compound or 2- or 3-hydroxymethylfuran compound or 2- or 3-hydroxymethyl thiophene compound according to the above formula (IIc) in the way as described above. The target cyclodepsipeptide product so produced by the intended condensation reaction may be recovered and obtained from the

resulting reaction solution in a manner known per se in the art.

The novel cyclodepsipeptide derivative of the formula (I) as produced by the above-mentioned processes may be formulated into a vermicidal or anthelmintic composition by mixing with an appropriate, pharmaceutically acceptable solid or liquid carrier.

According to a second aspect of this invention, therefore, there is provided an anthelmintic composition, characterized in that the composition comprises a cyclodepsipeptide of the general formula (I) as defined above or a non-toxic salt thereof, as an active ingredient, in combination with a solid or liquid carrier for the active ingredient.

The novel cyclodepsipeptide, PF1022 derivative of the formula (I) or a composition containing the same as the active ingredient according to this invention may be administered orally or parenterally to animals. The dosage of the compound of the formula (I) to be administered may suitably be determined by preliminary tests, depending upon the nature of parasitic organisms to be expelled, the nature of host animals in which parasites are living, and other several factors.

As the host animals to which can be given the novel cyclodepsipeptide, the PF1022 derivative of the formula (I) according to this invention as the vermicids, there are mentioned domestic animals, poultry, experimental animals

and companion animals such as swine, cattle, rabbit, sheep, goat, domestic fowl, turkey, mice, white rat, guiana pig, monkey, dog, cat, horse, small bird, and the like. Illustrative examples of parasites which live in these host animals include the parasites in cattle and sheep, such as twisted stomachworm, stomachworm belonging to the genus Ostertagia, small hairworm, nematodes belonging to the genus Cooperia, nodularworm belonging to the genus Oesophagostomum, amphisome, intestinal tapeworm (Moniezia benedeni), lung worm and liver fluke; the parasites on swine, such as roundworm, whipworm and nodularworm; the parasites on dog, such as roundworm, hookworm, whipworm and heart worm; the parasites on cat, such as roundworm and Spirometra mansoni; and the parasites on chicken, such as roundworm, hairworm and cecal worm. The new compound of this invention is also effective for the elimination of parasites on human bodies, such as roundworm, pinworm, hookworm (Ancylostoma duodenale, Ancylostoma ceylanicum, Necator Americanus), oriental hairworm, strongyloides worm and whipworm.

The novel cyclodepsipeptide, PF1022 derivative of the formula (I) according to this invention is particularly effective for the elimination of such parasites of the genus Trichostrongylus as classified into one of the hairworms living in the stomach of cattle and sheep, as well as for the elimination of parasites the genus Dirofilaria as classified into one of the heart worms living in the heart of dog and cat. These particular parasites can bring about

serious damages to the host animals.

From results of tests on anthelmintic activity of the compounds which are hereinafter given, it has been found that the novel PF1022 derivative of formula (I) according to this invention exhibits very much strong anthelmintic activities against Trichostrongylus colubriformis belonging to the genus Trichostrongylus, as well as against Trichinella spiralis belonging to the genus Trichinella (which is known to have a very close interrelation, in view-point of the anthelmintic effect, to the genus Dirofilaria), in comparison with the anthelmintic activity of the known PF1022 substance and the other known derivatives thereof.

The novel PF1022 derivative of formula (I) according to this invention is utilizable for the therapeutic treatment and prevention of parasitic infections. For the therapeutic treatment, the novel PF1022 derivative of formula (I) according to this invention may be administered orally and parenterally. For the oral administration, various methods are available, including such a method where a liquid preparation comprising said derivative is forcedly administered with using an implement such as stomach catheter; such a method where said derivative is mixed in any daily feed or drink water and the resulting mixture is administered, and such a method where said derivative is administered in the form of any appropriate preparation for usual oral administration such as tablets, capsules, pellets, boluses, powders, soft capsules, etc.

For the parenteral administration, the PF1022 derivative of formula (I) according to this invention may be administered by injections, subcutaneously, intramuscularly, intravenously, intraperitoneally, etc., in the form of a water-insoluble preparation comprising said derivative along with peanut oil, soybean oil, etc. or in the form of a water-soluble preparation comprising said derivative along with glycerol, polyethylene glycol, etc. In such parenteral preparations, the new compound of formula (I) according to this invention may, in general, be present in a range of 0.1 - 10% by weight.

For the prevention of parasitic infections, it is a common practice to administer the new compound of this invention orally in the form of a mixture with any daily feed.

No limitation is imposed on the administration period of the compound when the administration is done for preventive purposes. In most cases, it is sufficient to administer the active compound of formula (I) for about two months in broiler chickens, and for about five months in swine. The concentration of the active compound to be administered may be at least 1 ppm and preferably may be up to 5 - 10 ppm of the compound in the feedstock in which the new compound of formula (I) is mixed, and the administration may preferably be done continuously.

In a further aspect of this invention, there is provided the use of the cyclodepsipeptide of the general formula (I) as defined hereinbefore or a non-toxic salt

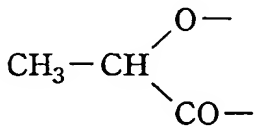
thereof, for the manufacture of an anthelmintic composition.

This invention is now concretely illustrated by the following Production Examples 1-11 and Evaluation Test Examples 1-4. The following abbreviations as used in the Production Examples denote the following meanings:

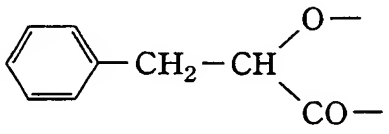
Me: Methyl group

Et: Ethyl group

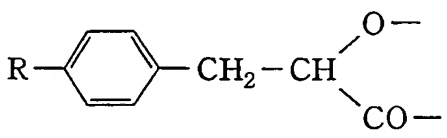
Lac: lactic acid residue represented by the formula



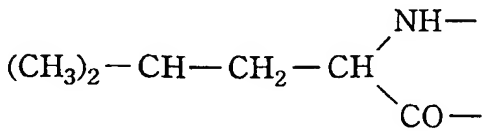
PhLac: phenyllactic acid residue represented by the formula



(R)PhLac: D-phenyllactic acid residue having a substituent, R group at the para-position of the benzene ring, which is represented by the formula

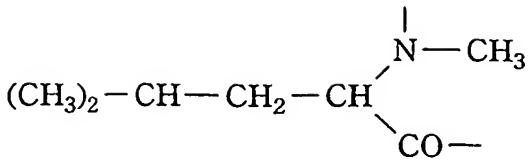


Leu: Leucine residue represented by the formula

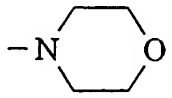


MeLeu: N-methylleucine residue represented by the

formula



Mor: Morpholino group of the formula



THF: Tetrahydrofuran

DMF: N,N-dimethylformamide

DEAD: Azodiacetic acid diethyl ester

Example 1 Production of Cyclo[MeLeu-Lac-MeLeu-(2-furylmethoxy)PhLac]$_2$

(Compound Code No.: PF1022-888 substance)

PF1022 H substance, namely Cyclo[MeLeu-Lac-MeLeu-(HO) PhLac]$_2$, (8.03 g), was dissolved in a mixture of acetone (240 ml) and DMF (60 ml), and to the resulting solution were added cesium carbonate (19.7 g), sodium iodide (4.23 g) and 2-chloromethylfuran (9.80 g). The resulting mixture was stirred at room temperature for 1.5 hours to effect the reaction. After the solvent was distilled off from the resulting reaction solution, ethyl acetate and water were added to the residue obtained. The resultant mixture was allowed to separate into liquid layers. The organic layer as separated was dried over magnesium sulfate and then the solvent was distilled off therefrom. The residue obtained was purified by a silica gel chromatography, followed by a reverse phase preparative high performance liquid

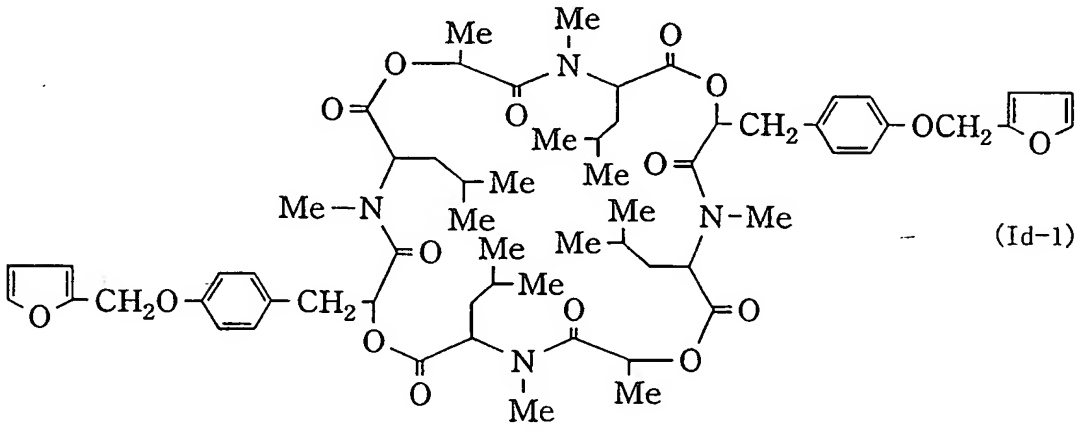
chromatography using ODS, thus to afford the titled compound (5.75 g; yield 62%), namely PF1022-888 substance which had the following data.

MS(FAB): 1141(M+H)

$[\alpha]_D$=-94.6° (MeOH, c=0.13)

$^1$H-NMR($CDCl_3$): $\delta$=0.80-1.05(m, 27H, $\delta$-H(MeLeu), $\beta$-H(Lac)), 1.35-1.84(m, 15H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu)), 2.73-3.14(m, 16H, N-Me(MeLeu), $\beta$-H(($(C_4H_3O)CH_2$)PhLac)), 4.44-4.50, 5.02-5.68 (each m, total 8H, $\alpha$-H), 4.96(s, 4H, ($(C_4H_3O)\underline{CH_2}$)PhLac)), 6.37-6.43(m, 4H, aromatic(furyl)), 6.87-6.92, 7.13-7.17 (each m, each 4H, aromatic(($(C_4H_3O)$-$CH_2$)PhLac)), 7.45(d, 2H, aromatic(furyl))

This PF1022-888 substance has the following structural formula (Id-1).



(Id-1)

Example 2 Production of Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(3-furylmethoxy)PhLac]

(Compound Code No.: PF1022-356 substance)

PF1022 E substance, namely Cyclo[MeLeu-Lac-MeLeu-

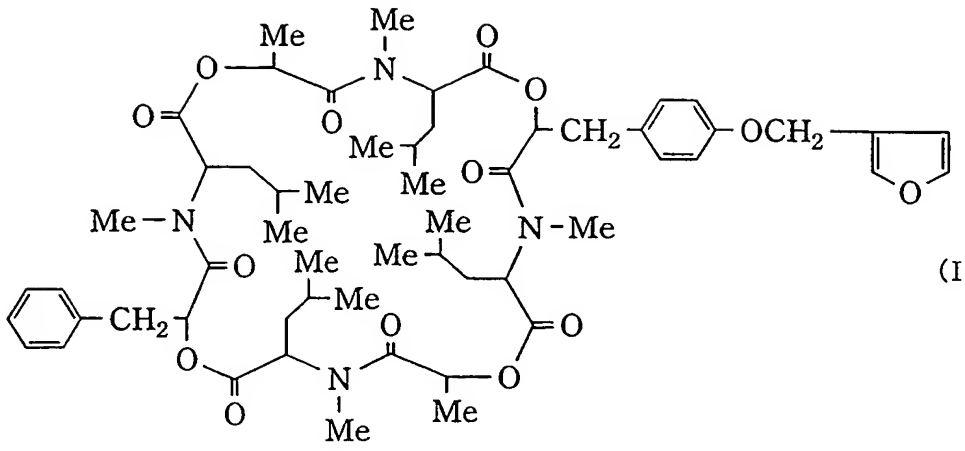
PhLac-MeLeu-Lac-MeLeu-(HO)PhLac], (302 mg) was dissolved in a mixture of acetone (10.0 ml) and DMF (3.0 ml), and to the resulting solution were added cesium carbonate (376 mg), sodium iodide (105 mg) and 3-chloromethylfuran (405 mg). The mixture so obtained was stirred at room temperature for 8.5 hours to effect the reaction. After the solvent was distilled off from the resulting reaction solution, ethyl acetate and water were added to the residue obtained. The resultant mixture was allowed to separate into liquid layers. The organic layer as separated was dried over magnesium sulfate and the solvent was distilled off therefrom. The resulting residue was purified by a silica gel chromatography, to afford the titled compound (241 mg; yield 74%), namely PF1022-356 substance which had the following data.

MS(FAB): 1045(M+H)

$[\alpha]_D$=-95.5° (MeOH, c=0.11)

$^1$H-NMR($CDCl_3$): $\delta$=0.77-1.08(m, 27H, $\delta$-H(MeLeu), $\beta$-H(Lac)), 1.35-1.81(m, 15H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu)), 2.67-3.17(m, 16H, N-Me(MeLeu), $\beta$-H(($(C_4H_3O)CH_2$)PhLac)), 4.44-4.49, 5.04-5.71(each m, total 8H, $\alpha$-H), 4.89(s, 2H, ($(C_4H_3O)\underline{CH_2}$)PhLac)), 6.47(m, 1H, aromatic(furyl)), 6.84-6.89, 7.12-7.31(each m, 2H, 7H, aromatic(($(C_4H_3O)$-$CH_2$)PhLac)), 7.41-7.43(m, 1H, aromatic(furyl)), 7.49(s, 1H, aromatic(furyl))

This PF1022-356 substance has the following structural formula(Ib-1).



(Ib-1)

Example 3 Production of Cyclo[MeLeu-Lac-MeLeu-(3-furylmethoxy)PhLac]$_2$

(Compound Code No.: PF1022-357 substance)

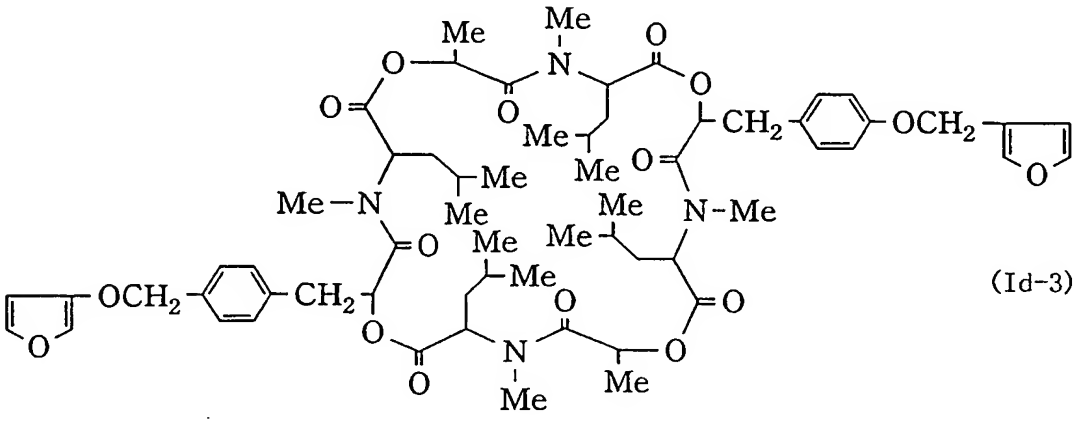
PF1022 H substance (365 mg) was dissolved in a mixture of acetone (10 ml) and DMF (3 ml), and to the resulting solution were added cesium carbonate (775 mg), sodium iodide (225 mg) and 3-chloromethylfuran (810 mg). The mixture so obtained was stirred at room temperature for 5 days to effect the reaction. After the solvent was distilled off from the reaction solution, ethyl acetate and water were added to the residue so obtained. The resultant mixture was allowed to separate into liquid layers. The organic layer separated was dried over magnesium sulfate and the solvent was distilled off therefrom. The residue obtained was purified by a silica gel chromatography, followed by a reverse phase preparative high performance liquid chromatography using DOS, thus to afford the titled compound (186 mg; yield 43%), namely PF1022-357 substance which had the following data.

MS(FAB): 1141(M+H)

$[\alpha]_D$=-94.6° (MeOH, c=0.13)

$^1$H-NMR($CDCl_3$): $\delta$=0.80-1.05(m, 27H, $\delta$-H(MeLeu), $\beta$-H(Lac)), 1.35-1.83(m, 15H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu)), 2.73-3.14(m, 16H, N-Me(MeLeu), $\beta$-H(($(C_4H_3O)CH_2$)PhLac)), 4.44-4.49, 5.03-5.65(each m, total 8H, $\alpha$-H), 4.89(s, 4H, ($(C_4H_3O)\underline{CH_2}$)-PhLac)), 6.47(m, 2H, aromatic(furyl)), 6.87-6.92, 7.13-7.17(each m, each 4H, aromatic(($(C_4H_3O)CH_2$)PhLac)), 6.84-6.89 7.12-7.17(each s, each 2H, aromatic(furyl))

This PF1022-357 substance has the following structural formula(Id-3).



(Id-3)

Example 4 Production of Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(2-thienylmethoxy)PhLac]

(Compound Code No.: PF1022-352 substance)

PF1022 E substance (413 mg) was dissolved in a mixture of acetone (10.0 ml) and DMF (3.0 ml), and to the resulting solution were added cesium carbonate (499 mg) and 2-chloromethylthiophene (185 mg). The mixture obtained was stirred at room temperature for 4 hours to effect the reaction. After the solvent was distilled off from the resulting

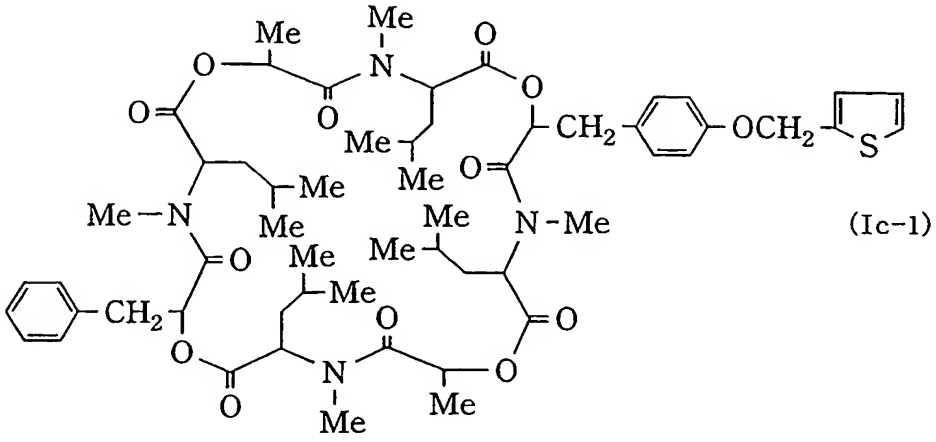
reaction solution, ethyl acetate and water were added to the residue. The resultant mixture was allowed to separate into liquid layers. The organic layer as separated was dried over magnesium sulfate and the solvent was distilled off therefrom. The residue obtained was purified by a silica gel chromatography, followed by a reverse phase preparative high performance liquid chromatography using ODS, thus to afford the titled compound (205 mg; yield 44%), namely PF1022-352 substance which had the following data.

MS(FAB): 1061(M+H)

$[\alpha]_D$=-82.3° (MeOH, c=0.18)

$^1$H-NMR($CDCl_3$): $\delta$=0.80-1.05(m, 27H, $\delta$-H(MeLeu), $\beta$-H(Lac)), 1.23-1.92(m, 15H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu)), 2.73-3.16(m, 16H, N-Me(MeLeu), $\beta$-H(($(C_4H_3S)CH_2$)PhLac)), 4.44-4.50, 5.03-5.70(each m, total 8H, $\alpha$-H), 5.18(s, 2H, (($(C_4H_3S)CH_2$)-PhLac)), 6.87-6.92, 7.13-7.17(each m, each 2H, aromatic($(C_4H_3S)CH_2$)PhLac), 7.22-7.29(m, 5H, aromatic(PhLac), 7.00, 7.10(each q, each H, aromatic(thienyl)), 7.31-7.34(m, 1H, aromatic(thienyl))

This PF1022-352 substance has the following structural formula (Ic-1).



(Ic-1)

Example 5 Production of Cyclo[MeLeu-Lac-MeLeu-(2-thienylmethoxy)PhLac]$_2$

(Compound Code No.: PF1022-353 substance)

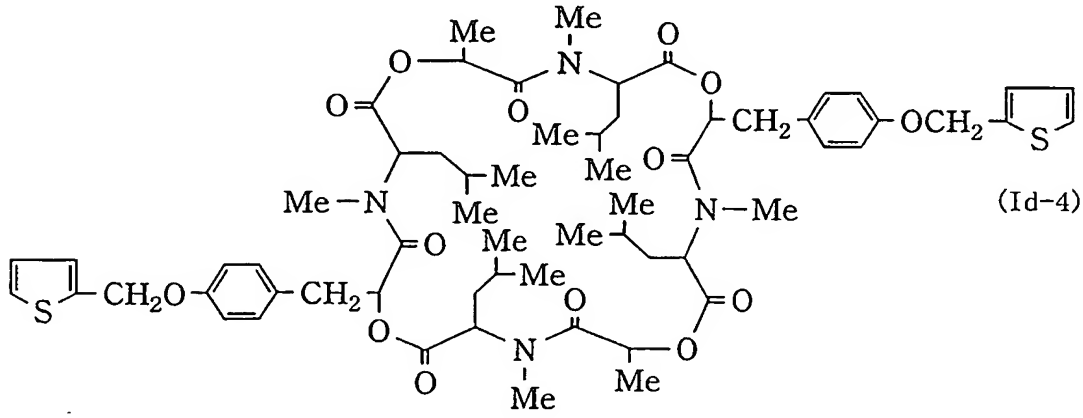
PF1022 H substance (423 mg) was dissolved in a mixture of acetone (10 ml) and DMF (3 ml), and to the resulting solution were added cesium carbonate (927 mg) and 2-chloromethylthiophene (9.80 g). The mixture obtained was stirred at room temperature for 20 hours to effect the reaction. After the solvent was distilled off from the reaction solution, ethyl acetate and water were added to the residue. The resultant mixture was allowed to separate into liquid layers. The organic layer as separated was dried over magnesium sulfate and the solvent was distilled off therefrom. The residue obtalued was purified by a silica gel chromatography, followed by a preparative high performance liquid chromatography using a reversed phase ODS, thus to afford the titled compound (54 mg; yield 11%), namely PF1022-353 substance which had the following data.

MS(FAB): 1173(M+H)

$[\alpha]_D$=-78.4° (MeOH, c=0.18)

$^1$H-NMR($CDCl_3$): $\delta$=0.80-1.05(m, 27H, $\delta$-H(MeLeu), $\beta$-H(Lac)), 1.35-1.81(m, 15H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu)), 2.73-3.09(m, 16H, N-Me(MeLeu), $\beta$-H(($(C_4H_3O)CH_2$)PhLac)), 4.44-4.50, 5.04-5.65(each m, total 8H, $\alpha$-H), 5.19(s, 4H, ($(C_4H_3S)CH_2$)PhLac), 6.37-6.43(m, 4H, aromatic(furyl)), 6.87-6.92, 7.13-7.18 (each m, each 4H, aromatic(($(C_4H_3S)CH_2$)PhLac)), 7.00, 7.10(each q, each 2H, aromatic(thienyl)), 7.31-7.34(m, 2H, aromatic(thienyl))

This PF1022-353 substance is represented by the following structural formula(Id-4).



(Id-4)

Example 6 Production of Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(5-ethoxycarbonyl-2-furylmethoxy)PhLac]

(Compound Code No.: PF1022-358 substance)

PF1022 E substance (200 mg) was dissolved in a mixture of acetone (7.0 ml) and DMF (2.0 ml), and to the resulting solution were added cesium carbonate (338 mg), sodium iodide (47 mg) and 5-ethoxycarbonyl-2-chloromethylfuran (0.16 ml). The mixture so obtained was stirred at room temperature for

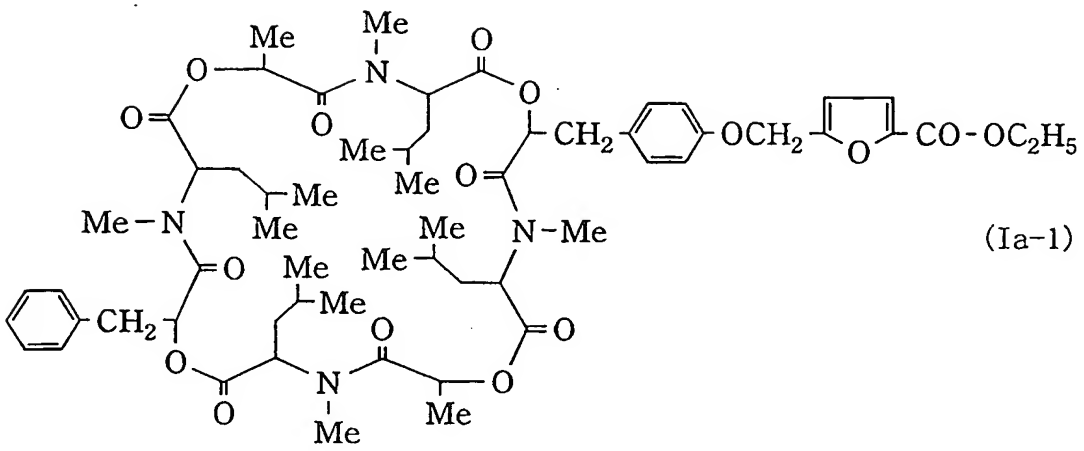
1 hour and 15 minutes to effect the reaction. After the resulting reaction solution was diluted with ethyl acetate, the diluted solution was washed twice with water. The organic layer as separated was dried over magnesium sulfate and then distilled to remove the solvent therefrom. The residue obtained was purified by a silica gel chromatography, affording the titled compound (223 mg; yield 95%), namely PF1022-358 substance.

MS(FAB): 1117(M+H)

$[\alpha]_D$=-84.7° (MeOH, c=0.21)

$^1$H-NMR($CDCl_3$): $\delta$=0.74-1.14(m, 27H, $\delta$-H(MeLeu), $\beta$-H(Lac)), 1.38-1.79(m, 18H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu), $COOCH_2\underline{CH_3}$), 2.74-3.16(m, 16H, N-Me(MeLeu), $\beta$-H((EtOCO($C_4H_2O$)$CH_2$)PhLac), PhLac), 4.36(q, 2H, $COO\underline{CH_2}CH_3$), 4.47-5.73(m, 8H, $\alpha$-H), 5.03(s, 2H, (EtOCO($C_4H_2O$)$\underline{CH_2}$)PhLac), 6.51(d, 1H, aromatic(furyl)), 6.87(d, 2H, aromatic((EtOCO($C_4H_2O$)$CH_2$)PhLac)), 7.14-7.29(m, 8H, aromatic(furyl,(EtOCO($C_4H_2O$)$CH_2$)PhLac, PhLac))

This PF1022-358 substance has the following structural formula(Ia-1).



(Ia-1)

Example 7 Production of Cyclo[MeLeu-Lac-MeLeu-(5-ethoxycarbonyl-2-furylmethoxy)PhLac]$_2$

(Compound Code No.: PF1022-359 substance)

PF1022 H substance (200 mg) was dissolved in a mixture of acetone (7.0 ml) and DMF (2.0 ml), and to the resulting solution were added cesium carbonate (398 mg), sodium iodide (92 mg) and 5-ethoxycarbonyl-2-chloromethylfuran (0.19 ml).

The mixture obtained was stirred at room temperature for 1 1/4 hours to effect the redction. After the resulting reaction solution was diluted with ethyl acetate, the diluted solution was washed twice with water. The organic layer as separated was dried over magnesium sulfate and the solvent was distilled off therefrom. The residue obtained was purified by a silica gel chromatography, to yield the titled compound (216 mg ; yield 84%), namely PF1022-359 substance.

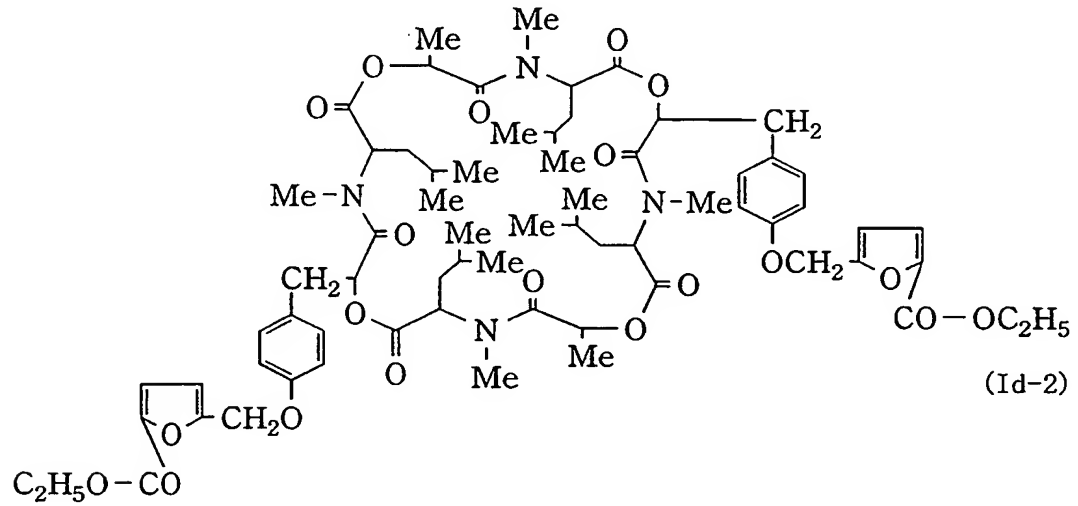
MS(FAB) : 1285(M+H)

$[\alpha]_D$=-89.4° (MeOH, c=0.22)

$^1$H-NMR($CDCl_3$) : $\delta$=0.77-1.07(m, 27H, $\delta$-H(MeLeu), $\beta$-H(Lac)), 1.39-1.72(m, 21H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu), $COOCH_2CH_3$), 2.74-3.10(m, 16H, N-Me(MeLeu),

β-H(((C4H3O)CH2)PhLac), PhLac), 4.37(q, 4H, COOCH2CH3), 4.47-5.65(m, 8H, α-H), 5.03(s, 4H, (EtOCO(C4H2O)CH2) PhLac, 6.51(d, 2H, aromatic(furyl)), 6.87(d, 4H, aromatic ((EtOCO(C4H2O)CH2)PhLac)), 7.16(m, 6H, aromatic(furyl, (EtOCO(C4H2O)CH2)PhLac))

This PF1022-359 substance is represented by the following structural formula(Id-2).



(Id-2)

Example 8 Production of Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(5-methyl-2-furylmethoxy)PhLac] (Compound Code No. : PF1022-360 substance)

(i) 5-Methyl-2-furaldehyde (1.0 ml) was dissolved in ethanol (20 ml), and to the resulting solution was added sodium borohydride(113 mg) under ice-cooling. The reaction was conducted under ice-cooling for 1 1/4 hours, then at room temperature for 6 hours, followed by adding another 57 mg portion of sodium borohydride to the reaction solution. The reaction was continued at room temperature for further 17 hours. After the solvent was distilled off from the

resulting reaction solution, methylene chloride and a 10% agueous sodium chloride solution were added to the resulting residue. The mixture so obtained was allowed to separate into liquid layers. The organic layer as separated was dried over anhydrous sodium sulfate and then concentrated to dryness, to yield 5-methyl-2-furfurylalcohol (1.0 g,yield 90%).

(ii) PF1022 E substance (400 mg) was dissolved in THF (8 ml), and to the resulting solution were added triphenylphosphine (543 mg) and 5-methyl-2-furfurylalcohol (279 mg) under ice-cooling and was then dropwise added DEAD(0.32 ml).

The reaction was conducted under ice-cooling for 2 hours. Isopropylether and ethyl acetate were added to the resulting reaction solution. The mixture obtained was filtered to remove insoluble triphenylphosphine oxide. The resulting filtrate was concentrated to dryness under a reduced pressure. The residue obtained was purified by a reverse phase preparative HPLC using ODS, thus to afford the titled compound (127 mg ; yield 29%) namely PF1022-360 substance.

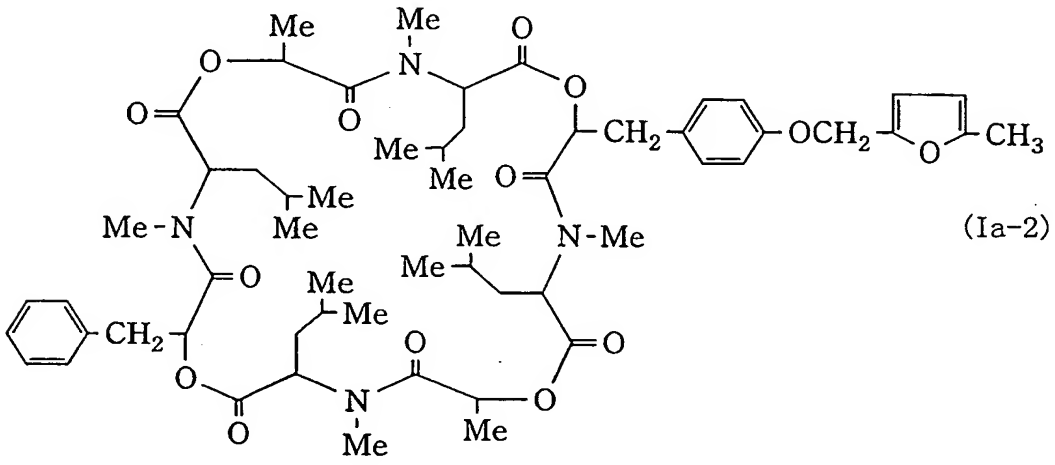
MS(FAB) : 1059(M+H)

$[\alpha]_D$=-91.7° (MeOH, c=0.22)

$^1$H-NMR($CDCl_3$) : $\delta$=0.75-1.17(m, 27H, $\delta$-H(MeLeu)), 1.39(m, 3H, $\beta$-H(Lac)), 1.47-1.84(m, 12H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu)), 2.31(s, 3H, Me($C_4H_2O$)$CH_2$), 2.72-3.23(m, 16H, N-Me(MeLeu), $\beta$-H((Me($C_4H_2O$)$CH_2$)PhLac)),

4.48-5.68(m, 8H, α-H), 4.90(s, 2H, (Me($C_4H_2O$)$\underline{CH_2}$)PhLac), 5.95, 6.30(each d, each 1H, aromatic(furyl)), 6.90, 7.15(each d, each 2H, aromatic((Me($C_4H_2O$)$CH_2O$)PhLac)), 7.20-7.37(m, 5H, PhLac)

This PF1022-360 substance has the following structural formula(Ia-2).



(Ia-2)

Example 9 Production of Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(5-ethyl-2-furylmethoxy)PhLac]

(Compound Code No. : PF1022-362 substance)

(i) 5-Ethyl-2-furaldehyde (920 mg) was dissolved in ethanol (20 ml), and to the resulting solution was added sodium borohydride(137 mg) under ice-cooling. The reaction was conducted at the same temperature for 1 hour. After the solvent was distilled off from the resulting reaction solution, methylene chloride and water were added to the residue. The mixture so obtained was allowed to be separate into liquid layers. The organic layer separated was dried over anhydrous sodium sulfate and then concentrated to dryness by removing the solvent. Thus was obtained 5-

ethyl-2-furfurylalcohol (901 mg ; yield 98%).

(ii) PF1022 E substance (500 mg) was dissolved in THF (10 ml), and to the resulting solution were added triphenylphosphine (408 mg) and 5-ethyl-2-furfurylalcohol (261 mg) under ice-cooling and was then dropwise added DEAD (0.24 ml).

The reaction was conducted under ice-cooling for 2 hours. Isopropylether and ethyl acetate were added to the resulting reaction solution. The mixture obtained was filtered to remove insoluble triphenylphosphine oxide. The resulting filtrate was concentrated to dryness under a reduced pressure. The residue obtained was purified by a reverse phase preparative HPLC using ODS, thus to afford the titled compound (170 mg ; yield 31%), namely PF1022-362 substance.

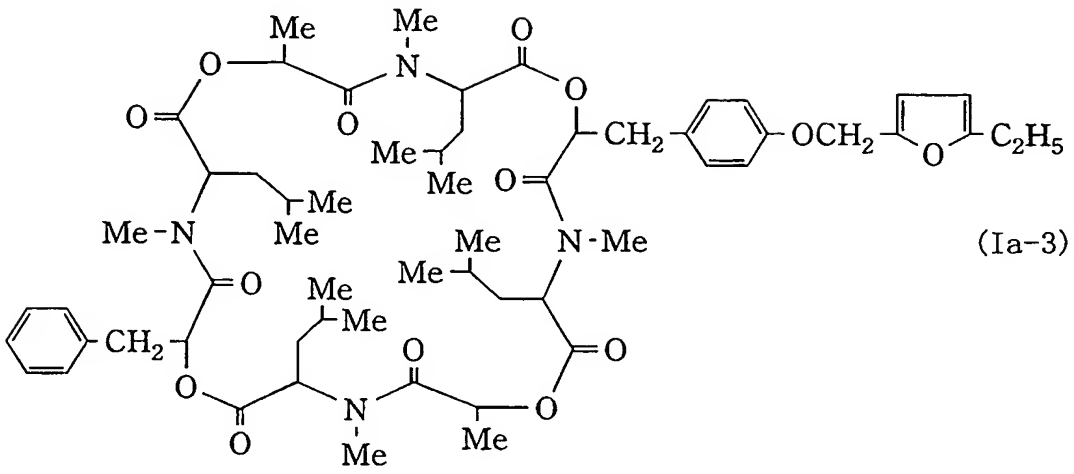
MS(TSI) : 1073(M+H)

$[\alpha]_D$=-85.8° (MeOH, c=0.20)

$^1$H-NMR($CDCl_3$) : $\delta$=0.75-1.15(m, 27H, $\delta$-H(MeLeu)), 1.23(t, 3H, $CH_2\underline{CH_3}$) 1.39(m, 3H, $\beta$-H(Lac)), 1.45-1.82(m, 12H, $\beta$-H(Lac), $\gamma$-H, $\beta$-H(MeLeu), 2.64(q, 2H, $\underline{CH_2}CH_3$), 2.72-3.21(m, 16H, N-Me(MeLeu), $\beta$-H((Et($C_4H_2O$)$\underline{CH_2}$)PhLac), PhLac), 4.48-5.67(m, 8H, $\alpha$-H), 4.91(s, 2H, (Et($C_4H_2O$)$CH_2$) PhLac), 5.96, 6.31(each d, each 1H, aromatic(furyl)), 6.90, 7.14(each d, each 2H, aromatic(Et($C_4H_2O$)$CH_2$)PhLac), 7.20-7.35(m, 5H, aromatic)

This PF1022-362 substance has the following

structural formula(Ia-3).



(Ia-3)

Example 10 Production of Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(4,5-dimethyl-2-furylmethoxy)PhLac]

(Compound Code No. : PF1022-364 substance)

(i) 4,5-Dimethyl-2-furaldehyde (1.02g) was dissolved in ethanol (20 ml), and to the resulting solution was added sodium borohydride(151 mg) under ice-cooling. The reaction was effected at the same temperature as above for 1 hour. After the solvent was distilled off from the reaction solution, methylene chloride and water were added to the residue. The mixture so obtained was allowed to separate into liquid layers. The organic layer as separated was dried over anhydrous sodium sulfate and then concentrated to dryness by removing the solvent. 4,5-Dimethyl-2-furfurylalcohol (1.01g ; yield 100%) was thus obtained.

(ii) PF1022 E substance (500 mg) was dissolved in THF (10 ml), to which were then added triphenylphosphine (408 mg) and 4,5-dimethyl-2-furfurylalcohol (261 mg) under ice-cooling and subsequently was dropwise added DEAD (0.24

ml).

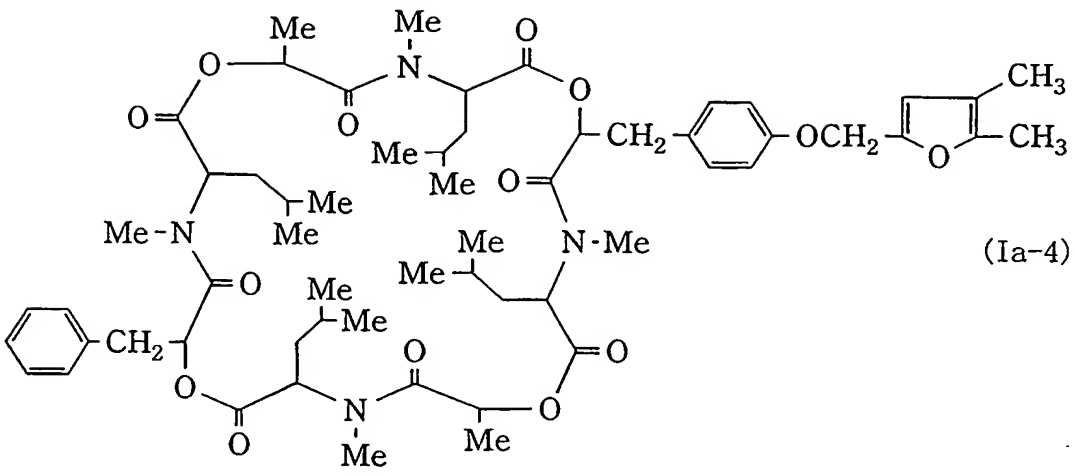
The reaction was effected under ice-cooling for 2 hours. Isopropylether and ethyl acetate were added to the resulting reaction solution. The mixture obtained was filtered to remove insoluble triphenylphosphine oxide. The filtrate obtained was concentrated to dryness under a reduced pressure, and the residue obtained was purified by a reverse phase preparative HPLC using ODS, thus to afford the titled compound (128 mg ; yield 23%), namely PF1022-364 substance.

MS(TSI) : 1073(M+H)

$[\alpha]_D$=-93.5° (MeOH, c=0.21)

$^1$H-NMR($CDCl_3$) : δ=0.74-1.08(m, 27H, δ-H(MeLeu)),
1.38(m, 3H, β-H(Lac)),
1.48-1.76(m, 12H, β-H(Lac), γ-H, β-H(MeLeu)),
1.93, 2.21(each s, each 3H, 4,5-dimethyl),
2.73-3.16(m, 16H, N-Me(MeLeu),
β-H(4,5-dimethyl-2-(furylmethoxy)PhLac), PhLac),
4.47-5.72(m, 8H, α-H),
4.85(s, 2H, (4,5-dimethyl-2-furyl) $\underline{CH_2}$O PhLac),
6.20(s, 1H, aromatic(furyl)),
6.89, 7.15(each d , each 2H, aromatic((4,5-dimethyl-2-furylmethoxy) PhLac)),
7.21-7.35(m, 5H, aromatic(PhLac))

This PF1022-364 substance has the following structural formula(Ia-4).



(Ia-4)

Example 11 Production of Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(5-(N,N-dimethylaminomethyl)-2-furylmethoxy)PhLac]

(Compound Code No. : PF1022-366 substance)

(i) 5-(N,N-Dimethylaminomethyl)furfurylalcohol hydrochloride (567 mg) was dissolved in methylene chloride (30 ml), and to the resulting solution was added a 7% aqueous sodium hydrogen carbonate solution under ice-cooling. The resultant mixture was allowed to separate into liquid layers. The organic layer as separated was dried over anhydrous sodium sulfate and then concentrated to dryness by removing the solvent. Thus, 5-(N,N-dimethylaminomethyl) furfurylalcohol (417 mg) was obtained.

(ii) PF1022 E substance (400 mg) was dissolved in THF (8 ml), and to the resulting solution were added triphenylphosphine (543 mg) and 5-(N,N-dimethylaminomethyl) furfurylalcohol (417 mg) under ice-cooling and then was dropwise added DEAD (0.32 ml).

The reaction was effected under ice-cooling for 3

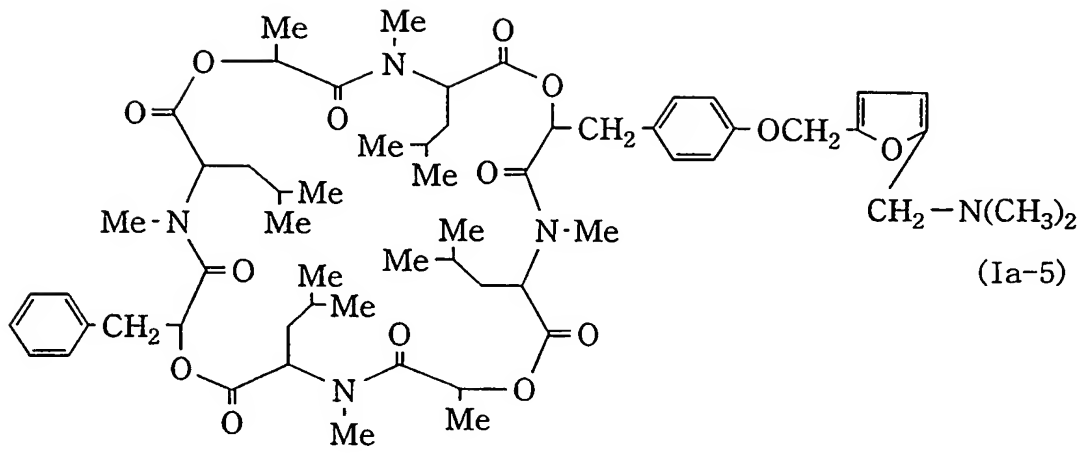
hours. Isopropylether and ethyl acetate were added to the resulting reaction solution, and the mixture obtained was filtered to remove insoluble triphenylphosphine oxide. The resultant filtrate was concentrated to dryness under a reduced pressure. The residues obtained was purified by a silica gel chromatography, thus affording the titled compound (164 mg ; yield 36%), namely PF1022-366 substance.

MS(TSI) : 1102(M+H)

$[\alpha]_D$=-89.1° (MeOH, c=0.23)

$^1$H-NMR($CDCl_3$) : $\delta$=0.70-1.05(m, 27H, $\delta$-H(MeLeu), $\beta$-H(Lac)), 1.26(m, 3H, $\beta$-H(Lac)), 1.47-1.78(m, 12H, $\gamma$-H, $\beta$-H(MeLeu)), 2.27(s, 6H, $NMe_2$), 2.73-3.16(m, 16H, N-Me(MeLeu), $\beta$-H(($Me_2$-$NCH_2$($C_4H_2O$)$CH_2$) PhLac), PhLac), 3.47(s, 2H, $Me_2NCH_2$), 4.48-5.71(m, 8H, $\alpha$-H), 4.93(s, 2H, ($Me_2NCH_2$($C_4H_2O$)$CH_2$) PhLac), 6.20, 6.35(each d, each 1H, aromatic(furyl)), 6.88, 7.14(each d , each 2H, aromatic($Me_2NCH_2$($C_4H_2O$)$CH_2$ PhLac)), 7.27(m, 5H, aromatic(PhLac))

This PF1022-366 substance has the following structural formula(Ia-5).

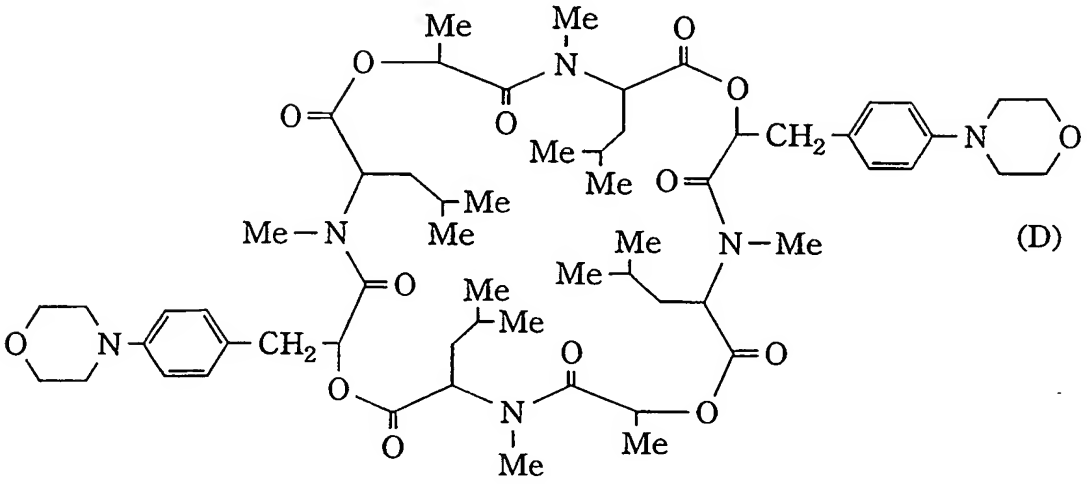


(Ia-5)

Further, the present inventors have now carried out some tests in animal in order to evaluate, in comparison, the anthelmintic activities of several novel cyclodepsipeptide derivatives of the formula (I) according to this invention, as well as the known PF1022 substance obtained as the fermentation product, a known morpholino derivative (namely, the known compound represented by cyclo [MeLeu-Lac-MeLeu-MorPhLac]$_2$ which is shown in Example 5 of the aforesaid PCT international publication WO93/19053), and PF1022-312 substance (namely, Cyclo[MeLeu-Lac-MeLeu-PhLac-MeLeu-Lac-MeLeu-(2-furylmethoxy)PhLac]) and PF1022-334 substance (namely, Cyclo[MeLeu-Lac-MeLeu-(2-pyridylmethoxy) PhLac]$_2$) (which are earlier synthesized by the present inventors and are described in Examples 24 and 42 of the specification of PCT international publication WO97/11064(pubtiahed 27 March 1997) of the aforesaid PCT/JP96/02730 application filed 20 September 1996).

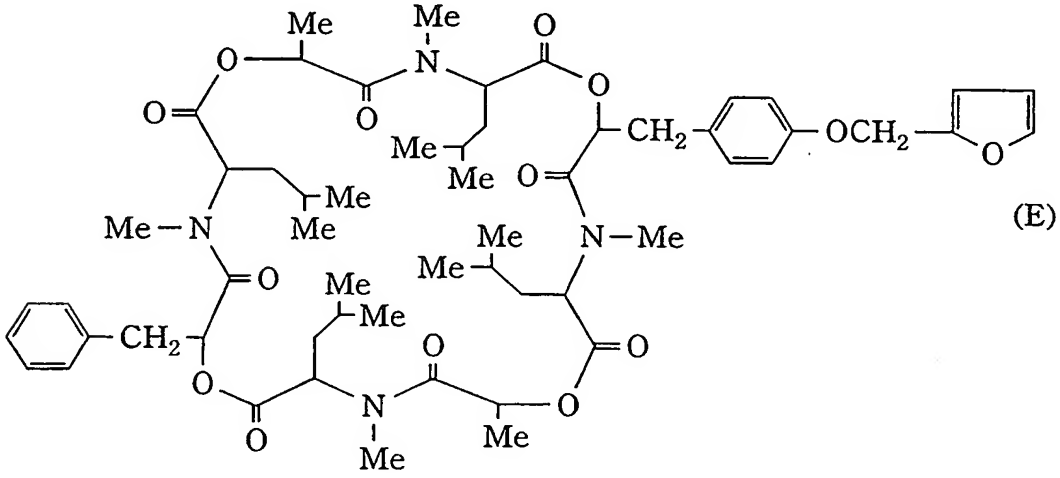
The above-mentioned known morpholino derivative tested as one of the comparative compounds is represented

by the following structural formula(D) :



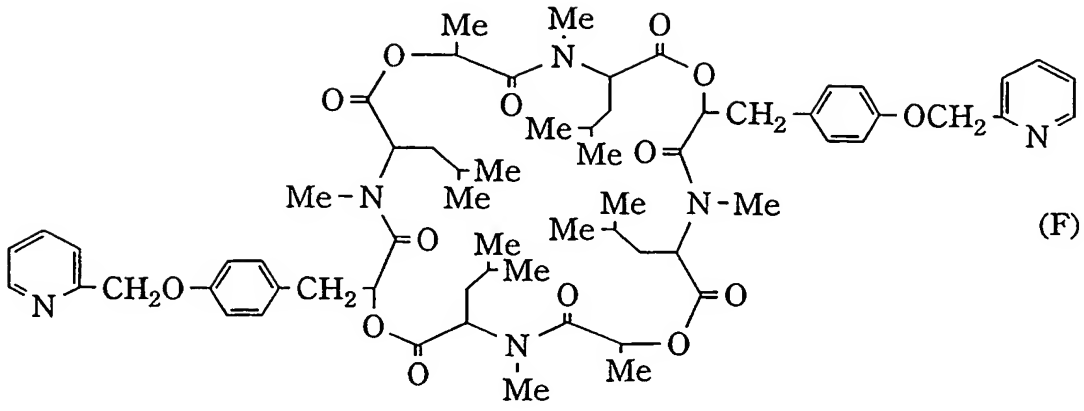
(D)

The above-mentiond PF1022-312 substance tested as another comparative compound has the following structural formula (E) :



(E)

The above-mentiond PF1022-334 substance tested as further comparative compound has the following structural formula (F) :



(F)

Evaluation Test Example 1 Tests in vivo of anthelmintic activity against Trichostrongylus colubriformis.

To each sheep which had been infected experimentally with a parasite, Trichostrongylus colubriformis, an anthelmintically active substance to be tested was orally administered in the form of gelatin caplules in a dosage (mg) of said test substance as accurately calculated on the basis of the body weight (kg) of the sheep. The number of the parasite's eggs which were excreted along with feces from the sheep was counted quantitatively before and after the administration of the test substance. Thereby, the degree of the anthelmintic effect obtained was evaluated. The test results are shown in Table 1 shown hereinafter. In Table 1 are indicated such dosages administered of the tested substances, including the novel PF1022 derivatives of the formula (I) according to this invention, at which no involvement of the excretion of the eggs could be observed, that is to say, the dosage at which a complete elimination of the test parasite from sheep could be achieved.

Thus, by this test, it has been demonstrated that the

tested novel PF1022 derivatives of this invention, namely PF1022-888, PF1022-358, PF1022-360 and PF1022-359 substances are able to exhibit such an excellently high anthelmintic activity that a complete elimination of the test parasite can be achieved by administration of the tested novel PF1022 derivatives of this invention at such low level of the dosages of administration which are 1/10 times to 1/25 times lower than the required dosage of the known PF1022 substance and which are 1/2 times to 1/5 times lower than the required dosage of the comparative, known morpholino derivative of the formula(D). In other words, the tested novel PF1022 derivatives of this invention can exhibit the anthelmintic activity of 10 times to 25 times higher than that of the known PF1022 substance and the anthelmintic activity of 2 times to 5 times higher than that of the known morpholino derivative.

Table 1

| Substance tested | Dosage administered (mg/kg) for 100% elimination of parasite |
|---|---|
| PF1022 substance (comparative) | 0.25 |
| The morpholino derivative (comparative) | 0.05 |
| PF1022-888 substance (of this invention) | 0.025 |
| PF1022-358 substance (of this invention) | 0.01 |
| PF1022-359 substance (of this invention) | 0.01 |
| PF1022-360 substance (of this invention) | 0.01 |

Evaluation Test Example 2 Tests in vivo of anthelmintic activity against Trichostrongylus colubriformis

To each sheep which had been experimentally infected with a parasite, Trichostrongylus colubriformis, an anthelmintically active substance to be tested was administered orally (per os) in such a dosage (mg) as accurately calculated on the basis of the body weight (kg) of the sheep.

The number of the parasite's eggs which were excreted along with feces from the sheep was counted quantitatively before and after the administration of the test substance. Thereby, the degree of the anthelmintic effect obtained was evaluated. The test results obtained are shown in Table 2 below. In Table 2, there are indicated such dosages of the tested substances, including the novel PF1022 substance of this invention, at which no involvement of the excretion of the parasite's eggs could be observed, that is to say, the dosages at which a complete elimination of the test parasite from sheep could be achieved.

Thus, it has been demonstrated by this test that the tested novel PF1022 substance of this invention, particularly PF1022-888 substance tested, is able to exhibit such an excellently high anthelmintic activity that a complete elimination of the test parasite can be achieved by administration of the tested novel PF1022 substance of this invention at such low level of the dosage of administration which is 1/ 10 times lower than the required dosage of the

known PF1022 substance and which is 1/2.5 times lower than the required dosage of the comparative, morpholino derivative of the formula (D). In other words, the tested PF1022-888 substance according to this invention can exhibit the anthelmintic activity of 10 times higher than that of the known PF1022 substance and the anthelmintic activity of 2.5 times higher than the comparative morpholino derivative which is a compound similar to PF1022 substance itself.

Table 2

| Substance tested | Dosage administered (mg/kg) for 100% elimination of parasite |
|---|---|
| PF1022 substance (comparative) | 1.0 |
| The morpholino derivative (comparative) | 0.25 |
| PF1022-888 substance (of this invention) | 0.1 |

Evaluation Test Example 3 Tests in vivo (per os) of anthelmintic activity against Trichinella spiralis

To each mouse which had been experimentally infected with a parasite, Trichinella spiralis, a substance to be tested was orally administered in such a dosage (mg) as accurately calculated on the basis of the body weight (kg) of the mouse.

The number of the parasite's eggs which were excreted along with feces from the mice was counted quantitatively before and after the administration of the test substance. Thereby, the degree of the anthelmintic effect obtained was evaluated. The test results are shown in Table 3 below. In

Table 3, there are indicated the rates(%) of efficacy which were calculated by making the comparisons between the counted number of parasite's eggs which were excreted from the mice as treated with each compound under test, and the counted number of the eggs which were excreted from the control mice which were equally infected with the parasite, but not treated with any compound (untreated group). The known PF1022 substance exhibited no anthelmintic activity at all against this particular parasite even at its dose of 100 mg. While, each of the known morpholino derivative as well as the earlier synthesized PF1022-312 substance of the formula (E) and PF1022-334 substance of the formula (F) had to be administered in increased dosages of 50 mg/kg or more in order to achieve a complete elimination of said particular parasite. In contrast thereto, it has been demonstrated that the novel PF1022 derivatives of this invention as tested are able to completely eliminate the test parasite when they were given at such a low dosage of 1 mg/kg or less, and thus that the new PF1022 derivatives of this invention exhibited a strong anthelmintic activity.

Table 3

| Substance tested | Dosage administered (mg/kg) | Efficacy Rate (%) |
|---|---|---|
| PF1022 substance (comparative) | 100 | 0 |
| The morpholino derivative (comparative) | 250 | 80 |
| | 100 | 50 |
| | 50 | 14 |
| | 25 | 3 |
| | 10 | 0 |
| PF1022-312 substance (comparative) | 50 | 40 |
| | 25 | 10 |
| | 10 | 0 |
| PF1022-334 substance (comparative) | 50 | 100 |
| | 25 | 50 |
| | 10 | 0 |
| PF1022-888 substance (of this invention) | 50 | 100 |
| | 10 | 100 |
| | 5 | 100 |
| | 1 | 100 |
| | 0.5 | 70 |
| | 0.25 | 0 |

Evaluation Test Example 4 Tests in vivo (i. p.) of anthelmintic activity against Trichinella spiralis

To each mouse which had been experimentally infected with a parasite, Trichinella spiralis, each substance to be tested was administered intraperitoneally (i.p.) in a dosage (mg) as accurately calculated on the basis of the body weight (kg) of the mouse.

The number of parasite's eggs which were excreted along with feces from the mice was counted quantitatively before and after the administration of the test substance. Thereby, the degree of anthelmintic effect obtained was

evaluated. The test results are shown in Table 4 below. In Table 4, there are indicated the rates(%) of efficacy which were calculated by making the comparison between the counted number of parasite's eggs which were excreted from the mice treated with each compound under test, and the counted number of the eggs which were excreted from the mice which were equally infected with the parasite but not treated with any test compound (untreated group). The known PF1022 substance and the known morpholino derivative exhibited no anthelmintic activity at all against this particular parasite even at their dose of 100 mg/kg. The comparative PF1022-312 substance had to be administered in a dosage of higher than 50 mg/kg in order to achieve a complete elimination of this parasite. In contrast thereto, it has been demonstrated that the tested PF1022-888 substance as one of the novel PF1022 derivatives according to this invention is able to completely eliminate this particular parasite at such a low dosage of 0.1 mg/kg or less, and thus that the novel PF1022-888 derivative of this invention exhibits a strong anthelmintic activity.

Table 4

| Substance tested | Dosage administered (mg/kg) | Efficacy Rate (%) |
|---|---|---|
| PF1022 substance (comparative) | 100 | 0 |
| Morpholino derivative (comparative) | 100 | 0 |
| PF1022-312 substance (comparative) | 50 | 90 |
| | 25 | 80 |
| | 10 | 20 |
| | 5 | 30 |
| PF1022-888 substance (of this invention) | 50 | 100 |
| | 10 | 100 |
| | 0.1 | 100 |

As is clear from the test results of the anthelmintic activity given above, the novel cyclodepsipeptide, PF1022 derivative of the formula (I) according to this invention exhibits a very strong and unique anthelmintic activity, as compared with the known PF1022 substance and the known derivatives thereof against Trichostrongylus colubriformis of the genus Trichostrongylus, as well as against Trichinella spiralis of the genus Trichinella which is known to have a good interrelation to the genus Dirofilaria in the viewpoint of anthelmintic effects. Besides, the novel PF1022 derivatives of this invention can be synthesized readily and efficiently by starting from the fermentatively produced PF1022 substance and its derivatives. Accordingly, the new PF1022 derivative of the formula (I) is very much useful as an anthelmintic agent.

As regards the safety of the novel cyclodepsipeptide PF1022 derivatives of the formula (I) of this invention, it

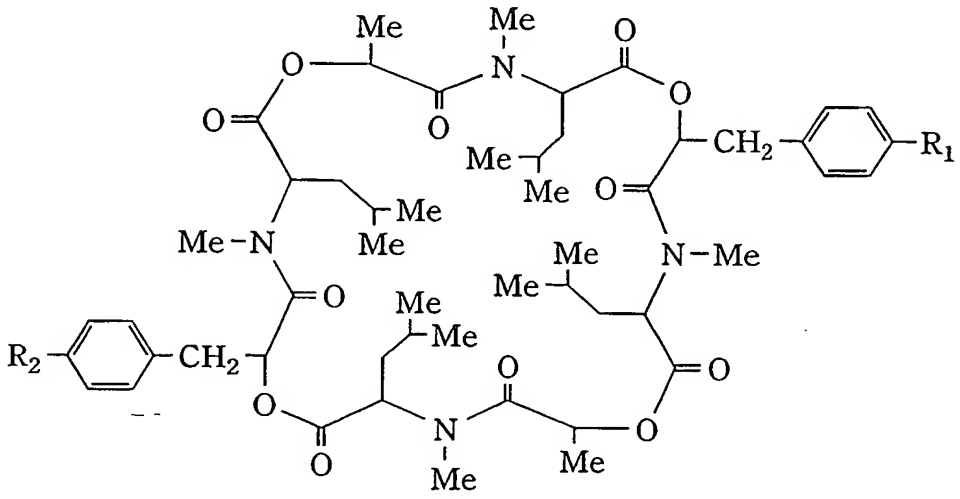
is shown that their administrations to mice at a dose of 300 mg/kg are accompanied by the normal increases in body weight of the mice and do not bring about any abnormality found in another aspects. This indicates that the novel PF1022 derivatives of this invention are of low toxicity.

Industrial Applicability

As will be clear from the foregoing descriptions, the novel cyclodepsipeptide, PF1022 derivatives according to this invention are easily synthesized from the fermentation product, PF1022 E or H substance and have an excellent anthelmintic activity, particularly against parasites of the genus Trichinella. So, they are very much useful as an anthelmintic agent.

CLAIMS

1. A cyclodepsipeptide represented by the following general formula (I)



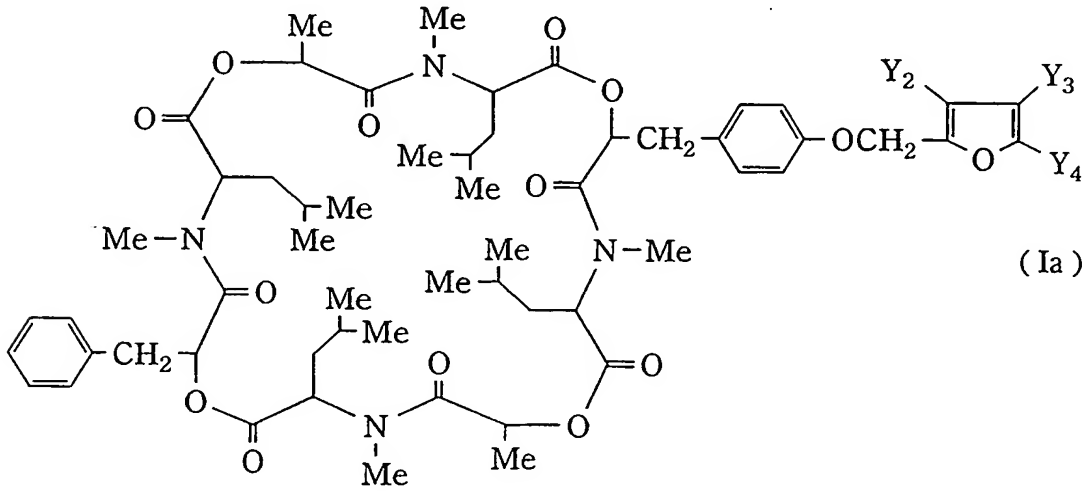
(I)

wherein Me denotes methyl group, and (i) $R_1$ is a hydrogen atom and $R_2$ is a 2-furylmethoxy group having one, two or three substituents on the furan ring, a 3-furylmethoxy group optionally having one, two or three substituents on the furan ring, a 2-thienylmethoxy group optionally having one, two or three substituents on the thiophene ring, or a 3-thienylmethoxy group optionally having one, two or three substituents on the thiophene ring, or alternatively (ii) both of $R_1$ and $R_2$ are identical to each other and are each a 2-furylmethoxy group optionally having one, two or three substituents on the furan ring, a 3-furylmethoxy group optionally having one, two or three substituents on the furan ring, a 2-thienylmethoxy group optionally having one, two or three substituents on the thiophene ring, or a 3-thienylmethoxy group optionally having one, two or three

substituents on the thiophene ring, or a non-toxic salt of said cyclodepsipeptide of the formula (I).

2. A cyclodepsipeptide as claimed in Claim 1, which is the cyclodepsipeptide of the general formula (I) where the one, two or three substituents on the furan ring or thiophene ring of the cyclodepsipeptide is or are each independently an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$) alkyl group substituted by an N-mono-($C_1$-$C_6$) alkylamino group, an N,N-di-($C_1$-$C_6$) alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$) alkoxycarbonyl group.

3. A cyclodepsipeptide as claimed in Claim 1, which is a cyclodepsipeptide represented by the following general formula (Ia)

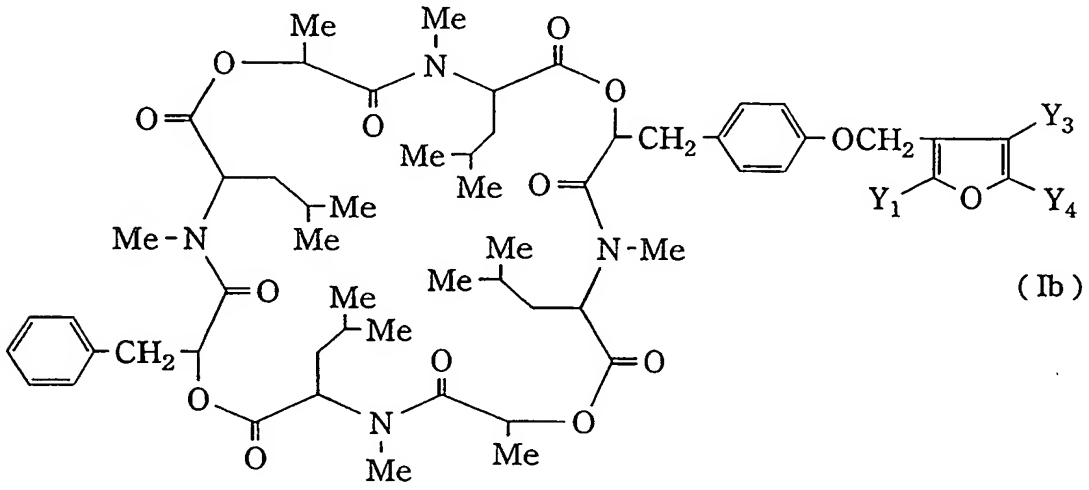


(Ia)

wherein Me is methyl group, and $Y_2$, $Y_3$ and $Y_4$ are each independently a substituent chosen from an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or

branched lower ($C_1$-$C_6$)alkyl group substituted by an N-mono-($C_1$-$C_6$)alkylamino group, an N,N-di-($C_1$-$C_6$)alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$) alkoxycarbonyl group, or a non-toxic salt of said cyclodepsipeptide.

4. A cyclodepsipeptide as claimed in Claim 1, which is a cyclodepsipeptide represented by the following general formula (Ib)

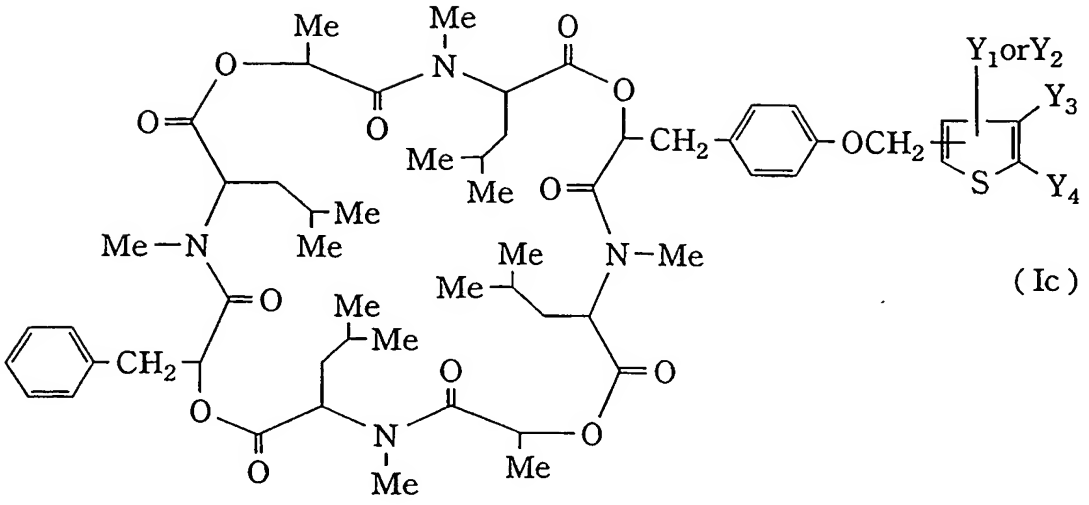


(Ib)

wherein Me is methyl group, and $Y_1$, $Y_3$ and $Y_4$ are each independently a hydrogen atom or a substituent chosen from an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$)alkyl group substituted by an N-mono-($C_1$-$C_6$)alkylamino group, an N,N-di-($C_1$-$C_6$) alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$) alkoxycarbonyl group, or a non-toxic salt of said cyclodepsipeptide.

5. A cyclodepsipeptide as claimed in Claim 1, which is a cyclodepsipeptide represented by the following general

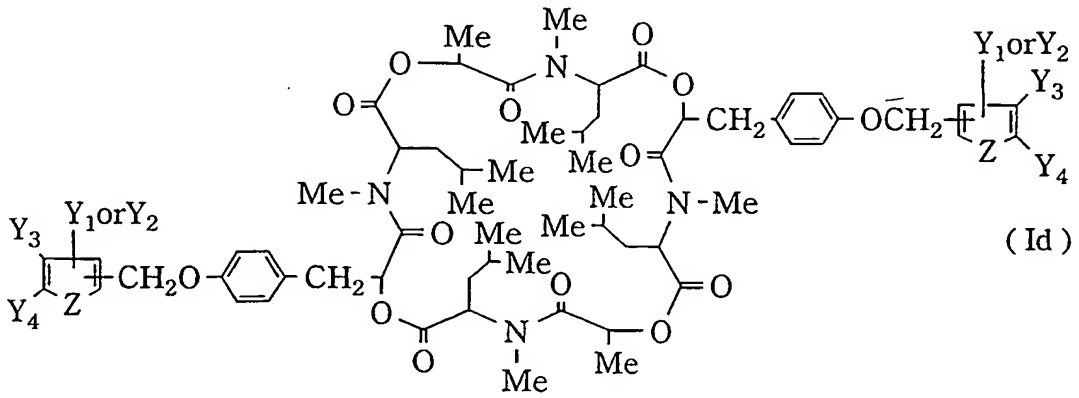
formula (Ic)



(Ic)

wherein Me is methyl group, and the existing $Y_1$ or $Y_2$, $Y_3$ and $Y_4$ are each independently a hydrogen atom or a substituent chosen from an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$)alkyl group substituted by an N-mono-($C_1$-$C_6$)alkylamino group, an N,N-di-($C_1$-$C_6$)alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$) alkoxycarbonyl group, or a non-toxic salt of said cyclodepsipeptide.

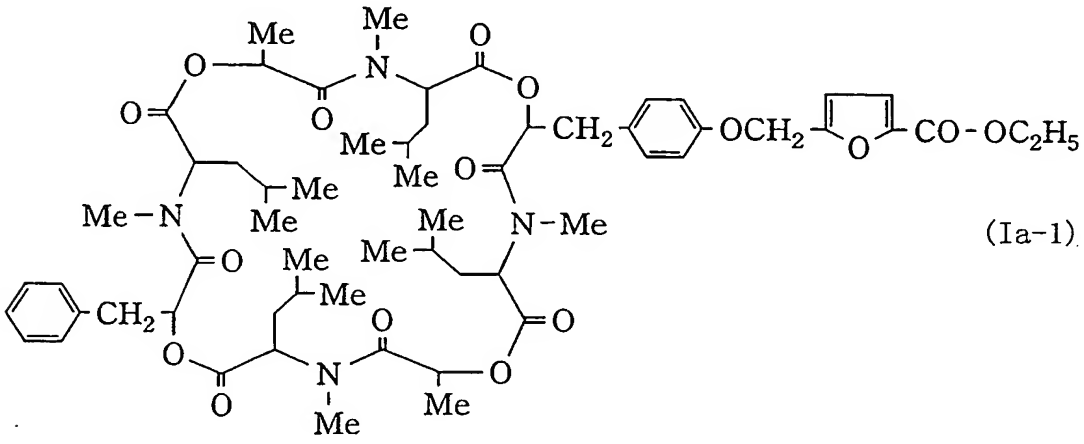
6. A cyclodepsipeptide as claimed in Claim 1, which is a cyclodepsipeptide represented by the following general formula (Id)



(Id)

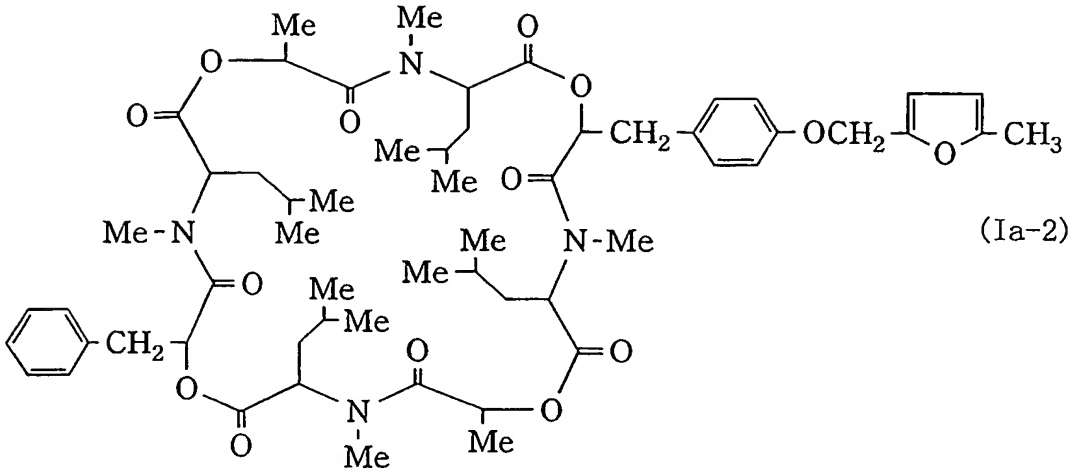
wherein Me is methyl group, Z is an oxygen atom or a sulfur atom, and the existing $Y_1$ or $Y_2$, $Y_3$ and $Y_4$ are each independently a hydrogen atom or a substituent chosen from an unsubstituted linear or branched lower ($C_1$-$C_6$) alkyl group, or a linear or branched lower ($C_1$-$C_6$)alkyl group substituted by an N-mono-($C_1$-$C_6$)alkylamino group, an N,N-di-($C_1$-$C_6$) alkylamino group, a ($C_1$-$C_6$)alkoxy group or a halo group, or a linear or branched lower ($C_1$-$C_6$)alkoxycarbonyl group, or a non-toxic salt of said cyclodepsipeptide.

7. A cyclodepsipeptide as claimed in Claim 3, which is a cyclodepsipeptide represented by the following formula (Ia-1)



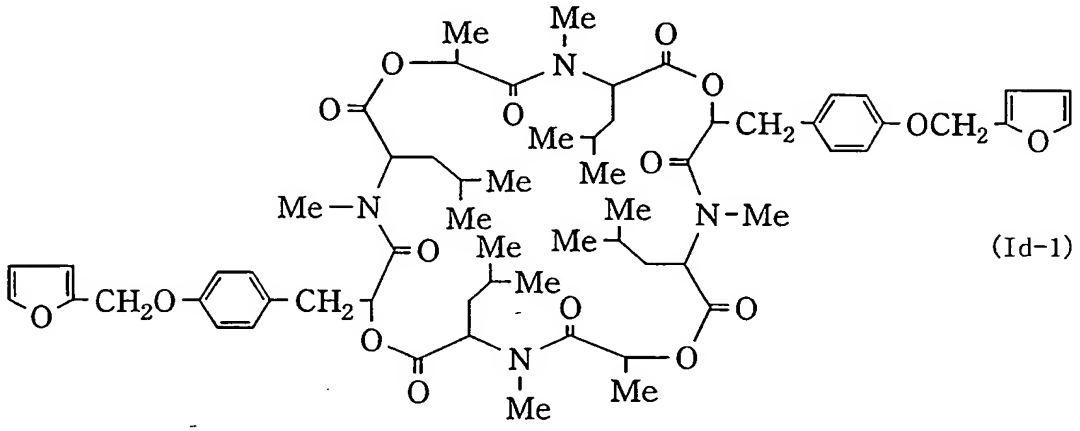
(Ia-1)

8. A cyclodepsipeptide as claimed in Claim 3, which is a cyclodepsipeptide represented by the following formula (Ia-2)



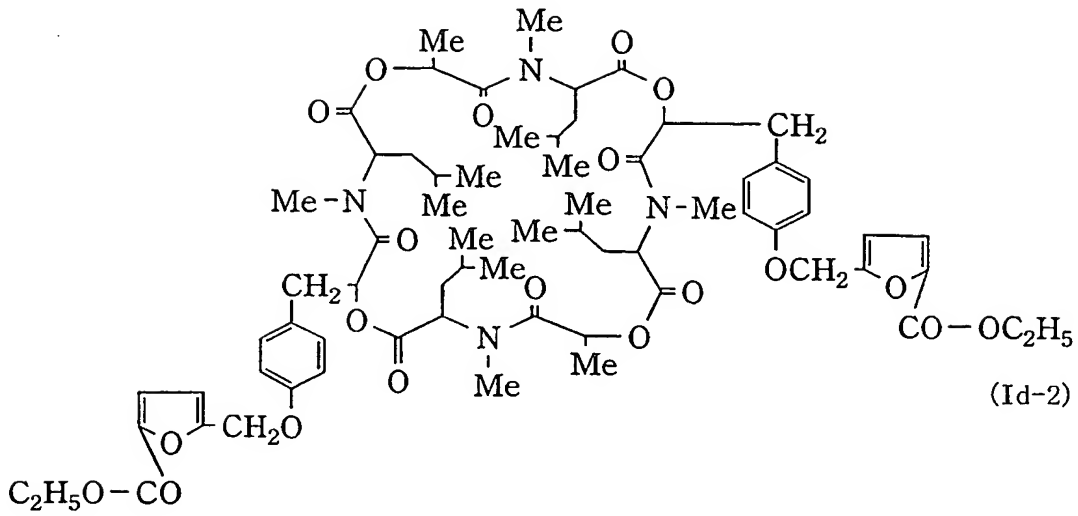
(Ia-2)

9. A cyclodepsipeptide as claimed in Claim 6, which is a cyclodepsipeptide represented by the following formula (Id-1)



(Id-1)

10. A cyclodepsipeptide as claimed in Claim 6, which is a cyclodepsipeptide represented by the following formula (Id-2)



(Id-2)

11. An anthelmintic composition, characterized in that the composition comprises a cyclodepsipeptide of the general formula (I) as defined in Claim 1 or a non-toxic salt thereof, as an active ingredient, in combination with a solid or liquid carrier for the active ingredient.

12. Use of the cyclodepsipeptide of the general formula (I) as claimed in Claim 1 or a non-toxic salt thereof, for the manufacture of an anthelmintic composition.

# INTERNATIONAL SEARCH REPORT

Inte [redacted] onal Application No
PCT/J[redacted]/00691

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K11/02 C07D273/00 A61K38/15

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A,P | WO 97 20945 A (BAYER AG ;JESCHKE PETER (DE); BONSE GERHARD (DE); THIELKING GERHAR) 12 June 1997 cited in the application whole document | 1-12 |
| P,X | WO 97 11064 A (MEIJI SEIKA KAISHA ;SAKANAKA OSAMU (JP); OKADA YUMIKO (JP); OHYAMA) 27 March 1997 whole document, esp. pages 1-28, 86-88, 103-105, 151-163, cf. esp. examples 24, 25,41 and 42 | 1-12 |
| A | WO 93 19053 A (FUJISAWA PHARMACEUTICAL CO) 30 September 1993 cited in the application whole document | 1-12 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 4 June 1998 | 15/06/1998 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Kronester-Frei, A |

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Inte ional Application No
PCT/JP 98/00691

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